Lipoprotein quality, anti-(xanthine oxidase) antibodies and coronary heart disease risk

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LIPOPROTEIN QUALITY, ANTI-(XANTHINE OXIDASE) ANTIBODIES AND CORONARY HEART DISEASE RISK

Submitted by

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ABSTRACT

This thesis involves two lines of investigation into aspects of coronary heart disease: firstly dyslipidaemia in non-insulin-dependent diabetes mellitus (NIDDM), systemic lupus erythematosus (SLE) and psoriatic arthritis (PA), and secondly the role and origin of human anti-xanthine oxidase (XO) antibodies.

Atherogenic abnormalities in fasting and postprandial lipemia were demonstrated in hypertriglyceridaemic subjects with NIDDM. Elevated fasting levels of large triglyceride-rich lipoproteins were exaggerated and prolonged postprandially in NIDDM compared to controls. Postprandial triglyceride response was proportional to fasting triglyceride levels in patients and controls. Raised levels of small, dense low density lipoprotein (LDL), reduced levels of high density lipoprotein (HDL) cholesterol and triglyceride-enrichment of LDL-2 and HDL persisted postprandially in NIDDM. Both fasting and postprandial triglyceride were strongly associated with prevalence of small, dense LDL in diabetic patients.

Bezafibrate therapy reduced fasting and postprandial triglyceride-rich lipoproteins and small, dense LDL in NIDDM towards that of normolipidaemic subjects. Reductions were greatest in chylomicrons and large very low density lipoprotein (VLDL). In the postprandial state, the proportion of large VLDL in total VLDL mass and triglyceride-enrichment of HDL were reduced in subjects with NIDDM. Such alterations clearly reduce atherogenic potential.

Fasting lipoprotein subfraction distribution and composition was studied in SLE and PA. In both patient groups, particularly females, the proportion of small, dense LDL in total LDL was elevated, independent of triglyceride levels. HDL cholesterol was significantly reduced in PA patients and tended to be low in SLE patients compared to controls. These abnormalities have atherogenic implications.

To study the origin and role of anti-xanthine oxidase antibodies, such antibodies were measured in the serum of normal healthy subjects. Levels of IgM anti-human xanthine oxidase (HXO) antibodies were higher than IgM anti-bovine xanthine oxidase (BXO) antibodies suggesting that the immunogen was endogenous HXO rather than ingested BXO. IgM anti-BXO (and HXO) antibodies were higher in younger females compared to age-matched males and older females. Levels of IgG anti-BXO and HXO were much lower and showed no such age and gender differences.

IgM anti-BXO antibody levels were significantly higher in patients dying within six months of a myocardial infarction (MI) compared to survivors and tended to be raised in patients who had suffered a MI compared to controls. No difference was found between

diabetic patients and controls. A lack of positive correlation of the extent of the MI with antibody levels suggests that these antibodies were not the result of the MI itself but produced prior to the MI, possibly due to chronic lesions of capillary endothelium exposing XO to the immune system. A protective role for anti-XO antibodies in heart disease, by neutralising XO, is consistent with the age and gender differences in healthy subjects.

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CONTENTS

ABBREVIATIONS

ACAT	acyl-Coenzyme A: cholesterol acyltransferase
Apo	apolipoprotein
ATP	adenosine triphosphate
AUC	area under curve
Beza	bezafibrate
BMI	body mass index
BXO	bovine xanthine oxidase
CCU	coronary care unit
CE	cholesterol ester
CETP	cholesteryl ester transfer protein
CFU	cumulative flotation ultracentrifugation
CH (chol)	cholesterol
CHD	coronary heart disease
Contr	controls
CPK	creatine phosphokinase
Dmax	maximum distance between cumulative distribution function
EC	esterified cholesterol
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme linked immuno-sorbent assay
FAD	flavin adenine dinucleotide
FC	free cholesterol
HbA1c	glycosylated haemoglobin
HDL	high density lipoprotein
HL	hepatic lipase
HMGCoA	3-hydroxy-3-methyl-glutaryl coenzyme A
HOMA	homeostasis model assessment
HXO	human xanthine oxidase
IAUC	incremental area under curve
IDDM	insulin-dependent diabetes mellitus
IDL	intermediate density lipoprotein
LCAT	lecithin: cholesterol acyltransferase
LDL	low density lipoprotein
Lp(a)	lipoprotein (a)

LPL	lipoprotein lipase
LRP	LDL receptor-related protein
MCD	maximum cumulative difference
MI	myocardial infarction
NAD(H)	nicotinamide adenine dinucleotide
NEFA	non-esterified fatty acids
NIDDM	non-insulin-dependent diabetes mellitus
PA	psoriatic arthritis
PL	phospholipid
Prot	protein
RA	rheumatoid arthritis
RCOOH	fatty acid
ROS	reactive oxygen species
SEM	standard error of the mean
S_f	svedberg unit of flotation
SH	sulphydryl group
SLE	systemic lupus erythematosus
TG (trig)	triglyceride
VLDL	very low density lipoprotein
XO	xanthine oxidase

CHEMICAL SYMBOLS

H_2O	water
H_2O_2	hydrogen peroxide
O_2	molecular oxygen
$O_2^{\cdot-}$	superoxide anion

CHAPTER 1

INTRODUCTION

This thesis separately investigates firstly dyslipidaemia in NIDDM, SLE and PA, and secondly the role and origin of human anti-xanthine oxidase antibodies in certain populations, in view of their potential role in atherogenesis and coronary heart disease. Chapter one includes an introduction to lipoprotein metabolism, lipoproteins and heart disease and dyslipidaemia in diabetes, systemic lupus erythematosus and psoriatic arthritis. Also included is an introduction to the properties, distribution, function and role in ischemia-reperfusion injury of xanthine oxidase, and finally the relationship between anti-xanthine oxidase antibodies and heart disease. The aims of the thesis are stated at the end of this Chapter. Methodology is described in Chapter 2 and results, with relevant discussion, are reported in Chapters 3-8. Conclusions are stated in Chapter 9.

1.1. CORONARY HEART DISEASE

Coronary heart disease (CHD) is the leading cause of death in economically advanced countries. In England and Wales 30% of all deaths amongst men and 22% of all deaths amongst women are the result of CHD (Dwyer et al, 1980), defined by the World Health Organisation as 'the cardiac disability, acute or chronic, arising from reduction or arrest of blood supply to the myocardium in association with disease processes in the coronary arterial system'. CHD in middle-age is predominantly a male problem, but gender differences are much less with increasing age (Heller, 1978). In women, oestrogen is believed to have a protective effect against CHD, which disappears after the onset of the menopause. There are marked international differences in the rate of occurrence of CHD. In general, the highest coronary mortality occurs in Northern Europe and Northern America, while in Southern Europe and Japan rates are much lower (Marmot, 1984). People who have migrated from a low-risk country, such as Japan, to a high-risk country, such as USA, have tended to adopt the rates of CHD of the high-risk country, suggesting that variations between countries are often due to environmental or behavioural differences (Nichamen et al.,1975). Known risk factors for CHD include hypercholesterolaemia, smoking, hypertension, diabetes mellitus, physical inactivity, age, male sex, family history of CHD and obesity.

In CHD, the reduction of flow of blood to the myocardium is caused by the narrowing of arteries supplying the tissue. This can be caused by thrombus formation, artery spasm or the thickening of the artery wall, a process known as atherosclerosis. There are three main clinical presentations of CHD:

1. Angina Pectoris. Usually a chronic and sometimes intermittent symptom, resulting from inadequate supply of oxygen to the myocardium. It is characterised by chest pain on exertion which is relieved by rest.
2. Myocardial Infarction. The sudden loss of sustained blood supply to the myocardium causes tissue injury and necrosis and prevents normal myocardial contraction. This is characterised by severe chest pain, and may or may not be fatal.
3. Sudden Death. Death from ventricular fibrillation may occur suddenly and without warning or within hours of the onset of cardiac symptoms.

Other manifestations of CHD include cardiac failure and non-fatal arrhythmias.

1.2. ATHEROSCLEROSIS

Atherosclerosis is defined as a variable combination of changes in the intima (inner lining) of arteries consisting of focal accumulation of lipids, other blood constituents and fibrous tissue, accompanied by changes in the media (middle layer) of the vessel wall. These features are the result of interaction between the structural and metabolic properties of the artery wall, composition of the blood and haemodynamic forces. Atherosclerosis develops over many years and may be asymptomatic. However, symptomatic atherosclerosis may occur in minutes and the clinical consequences can include coronary heart disease (angina pectoris, myocardial infarction and sudden death), cerebrovascular disease (stroke) or peripheral vascular disease (intermittent claudication, gangrene).

Endothelial damage, stemming from various causes including hypercholesterolaemia, is the initiating event in atherogenesis. Lipoproteins (LDL and VLDL/chylomicron remnants) become attached to the site of injury on the endothelial surface of the arteries and attract monocytes to the site of injury. These monocytes subsequently penetrate through the endothelium into the subintimal space and mature into macrophages. Macrophages become foam cells as they internalise lipid and accumulate resulting in formation of a fatty streak. Macrophages attract platelets to the site of the fatty steak which release platelet-derived growth factor causing smooth muscle proliferation, and the eventual conversion of a

fatty streak into a proliferative lesion or fibrous plaque, thus narrowing the lumen of the artery. The removal of lipoproteins from the plasma via macrophages is known as the scavenger pathway.

1.3. THE LIPOPROTEINS

1.3.1. Classification

Lipids are used by cells for a wide range of functions including membrane synthesis, hormone production and energy provision. The liver and intestine are the main sites of lipid synthesis and a directed and efficient transport mechanism has evolved in man and other species for their dispersion to other tissues. Neutral lipids (cholesterol esters and triglycerides) are hydrophobic and can only be transferred through the aqueous environment of plasma in macromolecular complexes containing protein (apolipoproteins) and polar lipids (phospholipids). These structures are known as lipoproteins and form a spectrum of particles which can be divided into a number of classes according to differing physical and/or chemical properties. The basic structure of lipoproteins consists of a hydrophobic core of triglyceride and cholesterol ester surrounded by a surface monolayer of protein and phospholipid. Free cholesterol spans the region intermediate between these two parts of the micelle structure.

The most common classification is based on hydrated density (g/ml) or their flotation rates, measured in Svedberg units (S_f) in the analytical centrifuge at a fixed background density. This approach was developed by Gofman, Lindgren and co-workers (Gofman et al, 1949) producing five classes of lipoprotein: chylomicrons, very low (VLDL), intermediate (IDL), low (LDL), and high (HDL) density lipoproteins, each of which is heterogenous in terms of size, lipid composition and apolipoprotein content. Techniques such as electrophoresis, gradient ultracentrifugation and affinity column chromatography have revealed structurally discrete subgroups within these classes. VLDL and LDL subfractions can be separated either by gel electrophoresis producing several bands of these lipoproteins, or by cumulative flotation ultracentrifugation (CFU) commonly producing three VLDL and three LDL subfractions. Some lipoproteins (VLDL and HDL) can also be isolated using precipitation techniques. Both the CFU and the precipitation techniques are used in this thesis and are described in more detail in the methods section (Chapter 2).

1.3.2. Composition

The physical and chemical properties of the major lipoprotein classes are shown in Table 1.1.

Chylomicrons are the largest lipoprotein particles varying in diameter from 75 to 1200nm. In normal individuals, chylomicrons are virtually absent in the fasting state. They are formed in the intestinal epithelial cells and their main lipid content, triglyceride, is synthesised from re-esterification of dietary monoglycerides and fatty acids. Triglycerides represent 80-90% by weight of chylomicrons and protein accounts for 1-2% of the mass. The remainder is composed of phospholipid, cholesterol ester and free cholesterol in decreasing proportion. They contain apolipoproteins B48, AI, AIV and C initially and apoCs and E are acquired from HDL or VLDL particles in the lymphatic or systemic circulation.

Very low density lipoproteins are similar to chylomicrons with triglyceride as their main lipid component. These particles are secreted by hepatocytes and range in size from 30-80nm with hydrated density <1.006g/ml. Apolipoproteins B100, CII, CIII and E are the main protein constituents and have important regulatory roles in the catabolism of these particles. β-VLDL are so called because they float at d<1.006g/ml upon ultracentrifugation but migrate with β-lipoproteins (LDL) on electrophoresis. β-VLDL are cholesterol-rich catabolic remnants of chylomicrons and VLDL, which are believed to be potentially atherogenic due to their receptor-mediated uptake by macrophages (Goldstein et al., 1980).

Intermediate density lipoproteins have a hydrated density of 1.006-1.019g/ml and represent a partial transition from VLDL to LDL particles as a result of the hydrolysis of the triglyceride moiety. The protein content of IDL is mainly apo B-100 and apo E with less apo C than VLDL.

Table 1.1. Physical and chemical properties of the major lipoprotein classes.

Lipoprotein	Density (g/ml)	Flotation rate (S_f)	Diameter (nm)	Electro-phoretic Mobility	Major apolipo-proteins	Esterified Cholesterol (%)	Free Cholesterol (%)	Triglyceride (%)	Phospho-lipid (%)	Protein (%)
Chylomicron	≤ 0.95	≥400	75-1200	Origin	B48, AI, E CII, CIII	5	2	84	7	2
VLDL	0.95-1.006	20-400	30-80	Pre-β	B100, CI, CII, CIII, E	11-14	5-8	44-60	20-30	4-11
IDL	1.006-1.019	12-20	25-35	Broad β	B100, E, CI, CII, CIII	22	8	30	25	15
LDL	1.019-1.063	0-12	18-25	β	B100	38	8	11	22	21
HDL	1.063-1.21	none	5-12	α	AI, AII, E, CI, CII, CIII	13	6	3	28	50

Flotation rate (S_f) is quoted against background solvent density of 1.063g/ml.

Low density lipoproteins are the main cholesterol-carrying lipoprotein in man and account for 70% of circulating cholesterol. These lipoproteins (hydrated density of 1.019-1.063g/ml) contain apo B100 as their major apolipoprotein and are formed by synthesis in the liver as well as catabolism of VLDL. Trace amounts of apo C and apo E are also present.

High density lipoproteins are the heaviest (density 1.063 to 1.21g/ml) and smallest (diameter 5.5-12.0nM) of the lipoproteins and contain apo AI and AII as their major protein moieties accounting for approximately 30% of the total protein. Trace amounts of apo CI, CII, CIII, D and E also present. HDL are the most abundant lipoprotein particles although they only carry 20% of the circulating cholesterol. They are formed from precursors synthesised in the liver and small intestine, or from the surface components of triglyceride-rich lipoproteins released during lipolysis. HDL are customarily divided into two major density classes: HDL_2 (density, 1.063-1.125g/ml) and HDL_3 (density, 1.125-1.21g/ml).

Lipoprotein (a) is an LDL-like particle, synthesised in the liver, containing cholesterol, phospholipid, triglycerides and apo B100, with the latter linked via a single disulphide bond to a second large protein termed apoprotein (a) (Utermann, 1989). The structure of apo (a) has a high degree of homology to plasminogen and contains a number of pretzel-like structures termed kringles. The molecular weight of apo (a) varies between 450-750KD, and the relationship between molecular weight and plasma concentration is an inverse one (Fless et al., 1985).

1.3.3. Apolipoproteins

The apolipoprotein moieties of plasma lipoproteins (Table 1.1) serve unique functions in the biogenesis, transport and metabolism of plasma lipoproteins. Eleven major apolipoproteins have been sequenced, and the genes responsible for their synthesis have been mapped in the human genome (Schonfeld, 1990; Breslow, 1991). With the exception of apo (a), the apolipoproteins possess an amphipathic helical region which contributes to their unique ability to transport lipids in an aqueous environment. Apolipoproteins (i) solubilise cholesterol esters and triglyceride by interacting with phospholipids; (ii) regulate the reaction of certain enzymes such as lecithin: cholesterol acyltransferase (LCAT), lipoprotein lipase (LPL) and hepatic lipase (HL); and (iii) bind to cell surface receptors therefore determining the sites of uptake and rates of degradation of their lipoprotein constituents. With the exception of apo B48, B100, and apo (a), all the apolipoproteins appear capable of dissociating from one lipoprotein and moving to another. This movement of apolipoproteins between lipoproteins not only serves to enhance processing of a given lipoprotein particle, but also prolongs the residence time of the apolipoproteins in plasma.

Apo AI is synthesised in the liver and intestine. It accounts for 70% of the total protein of HDL. Its main metabolic roles are as cofactor for the enzyme LCAT, and in promoting cholesterol efflux from the tissues. Apo AII is mainly synthesised in the liver and is the second commonest protein in HDL, although its function of has not been determined. Apo AIV is synthesised only in the small intestine and is a minor component of HDL and chylomicrons. It may play a role in the activation of LCAT.

Apo B48 is essential for the assembly and secretion of chylomicrons. It is so called because it constitutes 48% of the amino terminal end of apo B100 (Powell et al, 1987). It appears that apo B48 is generated from the same gene and messenger RNA as apo B100, but that only approximately half of the gene is translated into the protein in the intestine. Once the chylomicron has been secreted from the small intestine, it appears that apo B48 has no other role in the plasma metabolism of this lipoprotein or in the removal of chylomicrons from the plasma by the liver.

Apo B100 is the largest apolipoprotein (500KD), produced largely in the liver and secreted in VLDL particles on a 1:1 molar basis. It is essential for assembly and secretion of VLDL and as the ligand for the LDL receptor. The receptor binding domain of apo

B100 is absent from apo B48. Recent evidence suggests that variants of apo B100 which bind poorly to receptors may be an important cause of hyperlipidaemia in the population (Innerarity et al., 1990).

There are three C apolipoproteins (CI, II and III) all of which are synthesised in the liver. Apo CII has an important role as a cofactor for LPL which hydrolyses triglycerides in VLDL and chylomicrons, whereas Apo CIII is an inhibitor of LPL activity. The exact function of Apo CI is unknown.

Apo E is synthesised in the liver and is found on all the lipoproteins. Apo E on LDL and VLDL remnants bind to the LDL receptor. It regulates the removal of chylomicron remnants from the plasma by the liver, although the exact mechanism of this activity is unknown. The receptor responsible for the removal of these remnant particles appears to be distinct from the LDL receptor, and has apo E alone as its ligand (Hoeg et al., 1985).

As previously noted apo (a) is a protein associated with Lp (a). Apolipoprotein D is a minor component of HDL and its function is unknown.

1.4. LIPOPROTEIN METABOLISM

The wide variation in lipid and lipoprotein levels within and between populations suggests that the homeostatic mechanisms regulating plasma lipoprotein levels are not under tight control. Many factors can influence plasma lipoprotein levels including genes, hormones and diet. Abnormalities in lipoprotein metabolism are known to be associated with atherosclerosis and have been the subject of thorough investigation in recent years. The liver plays a crucial role in the homeostasis of lipoproteins by controlling their secretion and removal from the plasma. Lipoprotein metabolism can be considered as endogenous and exogenous pathways. The exogenous pathway is concerned with the absorption and transport of dietary fat and the endogenous pathway transports lipoproteins synthesised in the liver to the periphery.

1.4.1. The exogenous pathway

Nearly all the dietary cholesterol and triglyceride are transported by chylomicrons. Ingested triglycerides are hydrolysed in the intestine, releasing free fatty acids which are then re-esterified to form triglycerides in the intestinal mucosal cell and are assembled with apolipoprotein B48 and other apolipoproteins. During secretion from the enterocyte, these assembled particles enter the lymphatic circulation and then the blood stream where they acquire apo C and E from HDL and transfer apo AI and AII to HDL. In the plasma, the triglyceride is rapidly hydrolysed by lipoprotein lipase which resides on the surface of the capillary endothelium.

As triglyceride is hydrolysed from the chylomicrons, phospholipid, free cholesterol and apo A and C are transferred to HDL producing a triglyceride depleted and cholesterol enriched chylomicron remnant. These remnants are believed to be cleared by the liver by the binding of apo E to a protein similar to the LDL receptor (Beisiegel et al, 1989), called the LDL-receptor-related protein (LRP). The remnants enter liposomes in the liver from which cholesterol can enter metabolic pathways or be excreted into the bile.

1.4.2. The endogenous pathway

1.4.2.1. Very low density lipoprotein metabolism

VLDL are triglyceride-rich lipoproteins secreted by the liver and transport triglyceride to the peripheral tissues. Many factors influence the secretion of hepatic VLDL including nutrient intake, and plasma concentrations of free fatty acids, insulin, glucagon and epinephrine. The size of VLDL seems to be determined mainly by the amount of triglyceride available (Ginsberg et al., 1985). If triglyceride availability is reduced the liver may actually secrete IDL or LDL. VLDL are metabolised in a similar manner to chylomicrons by LPL, liberating fatty acids for uptake by the peripheral tissues. VLDL acquire additional apo C and E by transfer from HDL, in exchange for free cholesterol which is esterified by the enzyme LCAT and then returned to VLDL by cholesterol ester transfer protein (CETP) in exchange for triglyceride. As VLDL becomes triglyceride depleted, a portion of the surface including apo C and E and phospholipids are transferred to HDL. Smaller triglyceride-depleted VLDL particles (VLDL remnants or IDL) can either be removed from the circulation by receptors in the liver, including apo B/E receptors and

possibly LRP, or further metabolised by hepatic lipase (HL) to form LDL. Smaller more dense VLDL (S_f 20-60) are more efficiently converted to LDL via IDL, whereas larger VLDL (S_f 60-400) are more likely to be converted to a form of IDL which is removed by the liver (Packard et al., 1984).

1.4.2.2. Low density lipoprotein metabolism

LDL is the major cholesterol-carrying particle present in plasma and, metabolically, LDL may be regarded as the end product of VLDL metabolism. The only apolipoprotein present in LDL is apo B and only one molecule of apo B is present per particle. Clearance of LDL is mediated by a specific receptor (apo B/E or LDL receptor) present on the surface of both liver and peripheral cells. The identification of this receptor and its role in cholesterol homeostasis was recognised by Goldstein and Brown (1977) for which they were awarded a Nobel prize.

After binding to the receptor, the LDL are internalised and the influx of free cholesterol sets into motion a cascade of events to control the cholesterol content of the cell: (i) esterification of cholesterol is stimulated by activation of acyl-coenzyme A cholesterol acyl transferase (ACAT); (ii) de novo cholesterol synthesis is reduced by the inhibition of 3-hydroxy-3-methyl-glutaryl CoA (HMGCoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis; (iii) synthesis of the B/E receptor is inhibited which therefore limits further uptake of LDL cholesterol. Although the numbers and activity of LDL receptors are major factors in determining plasma LDL cholesterol levels, the initial rates of entry of VLDL into plasma and the efficiency with which VLDL are converted to LDL play crucial roles in determining steady-state LDL concentrations in the plasma.

The scavenger pathway is an alternative mechanism for the removal of LDL from the circulation. This mechanism is not mediated by the B/E receptor but by the scavenger receptor on tissue macrophages as well as by non-specific endocytotic uptake. These processes become increasingly important when either defects in the LDL receptor or abnormalities in VLDL and LDL composition are present.

1.4.2.3. High density lipoprotein metabolism

HDL is thought to play a critical role in preventing tissue accumulation of cholesterol. Nascent HDL are secreted by the liver as cholesterol-poor, protein-rich particles containing apo C, E and A, although some HDL is produced by the intestine. HDL particles can contain apo AI and AII or just AI alone and because of the varying amounts of cholesterol per HDL particle, measurements of apo AI in plasma provide a better indication of HDL particle number than measurements of HDL cholesterol. Surface components of chylomicrons and VLDL are transferred to HDL during lipolysis. Cholesterol is transferred from peripheral cell membranes to HDL and is subsequently esterified by LCAT, which circulates in association with HDL. In this way, the small dense HDL precursor, known as HDL_3, increases in size during the lipolytic process and cholesterol transfer process producing larger particles known as HDL_2. The esterified cholesterol can either be stored within the non-polar core of the particle or transferred to triglyceride-rich lipoproteins by CETP in exchange for triglyceride. HDL_2 as well as triglyceride-rich lipoproteins are cleared by the liver and therefore assist the pathway for reverse cholesterol transport by which cholesterol from the peripheral tissues can be removed and transported to the liver for excretion in the bile. Triglyceride-rich HDL_2 is hydrolysed by hepatic lipase producing HDL_3. In addition, hepatic lipase may hydrolyse HDL phospholipids, which in turn may promote the transfer of cholesterol from the HDL surface to the liver. Finally, there may be specific HDL receptors on peripheral cells that may be involved in the specific uptake and degradation of the HDL particles (Graham et al, 1987).

1.4.2.4. Lipoprotein (a) metabolism

Lp (a) is synthesised in the liver. Little is known about its clearance, but it is believed to be cleared by the B/E receptor. Increased plasma Lp(a) concentrations are associated within increased risk of cardiovascular disease (Hoefler et al, 1988), but the precise physiological function of Lp (a) is unknown (Kostner et al, 1992).

1.5. LIPOPROTEINS AND HEART DISEASE

Total plasma or serum cholesterol levels have been shown to correlate with CHD in both case-control and prospective studies (Nichamen et al.,1975; Keys, 1980; Martin et al.,1986; Stamler et al.,1986; Shaper et al., 1986). Initially it was believed that a threshold level existed below which the risk of CHD reached a plateau, but a more recent study suggests that the risk is continuous over the whole range of serum cholesterol (Martin et al.,1986). This study showed that risk of CHD rises appreciably when cholesterol levels exceed 6.5 mmol/l and even more so when levels exceed 7.8 mmol/l. Lowest rates of CHD occurred in individuals with serum cholesterol levels of below 5.2 mmol/l. Although hypercholesterolaemia alone is certainly a major factor for CHD risk, there are a variety of processes in lipoprotein metabolism which also play an important role in atherogenesis.

1.5.1. Chylomicrons

Abnormalities in transport and metabolism may lead to the involvement of chylomicrons in the atherogenic process (Zilversmit, 1979). Situations where prolonged residence times of chylomicrons might occur would include states where there is reduced LPL activity (eg. diabetes) or where there is an imbalance of apo CII and CIII. Excess VLDL could also compete with chylomicrons for LPL resulting in elevated chylomicrons (Brunzell et al, 1973). Several studies have associated abnormalities of chylomicrons or chylomicron remnant metabolism with the presence of CHD (Groot et al., 1991; Simons et al., 1987). In these studies men with CHD have higher postprandial levels of chylomicron triglycerides than men without the disease.

1.5.2. Very low density lipoproteins

Fasting triglyceride levels are among the most potent risk factors for CHD. In fact, hypertriglyceridaemia is one of a number of CHD risk factors in a constellation known as Syndrome X. This syndrome comprises of insulin resistance, glucose intolerance or diabetes, obesity, hypertriglyceridaemia, low HDL cholesterol, and hypertension. However, in multivariate analyses including other lipid parameters, the association between fasting

triglyceride and CHD is often not maintained, primarily because HDL cholesterol, by virtue of its inverse association with triglycerides, eliminates triglyceride as a risk factor for CHD (Austin, 1991). Elimination through statistical analysis constitutes a paradox because in reality, the concentration of HDL is dependent on the metabolism of triglyceride-rich lipoproteins (Tall, 1990), and multivariate analysis requires an independent variable. The heterogenous nature of VLDL reduces the likelihood that simply measuring triglyceride levels will predict risk. VLDL consist of a large range of particle sizes, depending on the triglyceride content of the particle, however, contrary to measuring serum and VLDL triglyceride, there is not a quick and simple method for measuring concentrations of VLDL subfractions.

Large triglyceride-rich VLDL particles, not abundant in normal subjects, can undergo cholesterol ester transfer thus becoming cholesterol ester-enriched. These VLDL remnants, as well as chylomicron remnants, are also known as β-VLDL and can be removed by the scavenger pathway, taken up by receptors on macrophages (Goldstein et al., 1980) resulting in foam cell formation. A β-VLDL receptor has been identified in macrophages which is specific for chylomicrons, large VLDL particles from hypertriglyceridaemic subjects and the β-migrating VLDL of patients with hypertriglyceridaemia (Mahley et al., 1984). More recently, it has been shown that the uptake of hypertriglyceridaemic, large VLDL by macrophages was higher than for normal VLDL and that these hypertriglyceridaemic VLDL populations were enriched with cholesteryl ester and apo E (Sehayek et al., 1990). Some evidence suggests that chylomicrons and hypertriglyceridaemic VLDL may have a unique receptor on macrophages which does not require apo E for uptake. (Gianturco et al., 1989, Gianturco et al., 1990).

Hypertriglyceridaemia has major effects on the composition and function of LDL and HDL (Eisenberg et al., 1984), which accordingly could influence their metabolism. Predominance of small dense LDL and reduced levels of HDL are commonly found in individuals with increased levels of VLDL (Austin and Krauss, 1986; Austin et al., 1988). Furthermore, low HDL cholesterol have been shown to be associated with CHD risk (Hulley et al.,1980) and predominance of small dense LDL appears to be a strong marker for the risk of premature CHD (Austin et al, 1988). Triglyceride transfers to HDL and LDL via CETP in exchange for cholesterol ester (Tall, 1986). LDL enriched with triglyceride is substrate for hydrolysis by lipases which results in a continuous particle size reduction to produce small, dense cholesterol and triglyceride depleted LDL (LDL-3) (Auwerx et al., 1988; Levy et al., 1990). Recent evidence suggests that hepatic lipase is responsible for this hydrolysis (Zambon et al., 1993). Kinetic studies of LDL metabolism suggest that small

dense LDL are derived from large triglyceride-rich VLDL in the hypertriglyceridaemic state, whereas in the normotriglyceridaemic state, the liver produces smaller VLDL particles which give rise to larger, more buoyant LDL (LDL-1 and -2) (Caslake et al., 1992). The transfer of cholesterol ester from HDL partly accounts for the reduced HDL cholesterol levels found in individuals with elevated levels of triglyceride and small, dense LDL.

Most of life is spent in the postprandial state in the western world, and although most studies have measured triglycerides in the fasting state only, it has recently been shown that postprandial triglyceride levels exhibited an association with CAD that was statistically independent and stronger than that of HDL cholesterol (Patsch et al., 1992). In the postprandial state and in hypertriglyceridaemia, the atherogenic potential of triglyceride-rich lipoproteins, including production of cholesterol ester-rich VLDL remnants, low HDL cholesterol and a preponderance of small, dense LDL, is exacerbated.

1.5.3. Low density lipoproteins

LDL is the main cholesterol-bearing lipoprotein in plasma and has been the one most consistently related to CHD (Grundy, 1986). Cholesterol is provided from endogenous synthesis in the liver and from the diet. In man, the levels of endogenous LDL secreted exceed that required by the peripheral tissues, to suppress cell HMGCoA reductase and therefore to regulate cholesterol synthesis. However, failure of this feedback mechanism can result in elevated LDL cholesterol levels, resulting in atherogenesis. For example, the number of functional LDL receptors plays an important role in the condition of familial hypercholesterolaemia (Goldstein and Brown, 1983). This condition is the result of single gene defects affecting the production and function of the LDL receptor. Heterozygotes have only half the normal number of functional receptors resulting in approximate doubling of plasma cholesterol; homozygotes have virtually no functional receptors and have 3-4 times the normal plasma cholesterol levels. The latter often die from CHD before the age of twenty. Familial hypercholesterolaemia occurs in 0.2% of the population and therefore deficient regulation of LDL metabolism by the LDL receptor cannot be responsible for atherosclerosis in the general population. This has led investigators to search for qualitative alterations in LDL that might play an important role in atherosclerosis.

Monocytes not only possess LDL receptors but also receptors for various forms of modified LDL. Unlike LDL receptors, these receptors (known as acetyl LDL receptors or scavenger receptors) are not down-regulated by accumulation of cholesterol within the

macrophage. One modification of LDL is peroxidation of phospholipids and partial degradation of apo B producing oxidised LDL which alters and reduces its interaction with the LDL receptor, and instead is avidly taken up by the modified LDL receptor (Steinberg et al., 1987). Oxidised LDL is more atherogenic than normal LDL in that it is chemotactic for monocytes (Quin et al., 1987), it inhibits mobility of tissue macrophages (Quin et al., 1985), and it is cytotoxic causing damage to endothelial cells (Henrikson et al., 1979). The accumulation of oxidatively modified LDL in macrophages results in the formation of foam cells which promote the progression of a fibrous plaque. Other modifications of LDL that lead to increased uptake by macrophages include acetylation (Goldstein et al., 1979) and glycation (Lyons et al., 1987).

There is also increasing evidence that subpopulations of LDL exist that differ in lipid composition, density and size and which may have differing atherogenic potentials. Small, dense LDL appear to be a strong marker for the risk of developing premature CHD (Austin et al., 1988). The LDL profile is commonly classified into two LDL phenotypes based on separation by gel electrophoresis: Phenotype A, (predominance of large light LDL), and Phenotype B (predominance of small dense LDL). Thirty to thirty-five percent of the adult male population have type B phenotype, however, the LDL phenotype B is less frequent in young males (<20 yrs) and premenopausal females (Austin et al., 1990; Campos et al., 1992a), with a slightly higher prevalence in postmenopausal women (Campos et al., 1992a; Selby et al., 1993). Complex segregation analysis has indicated heritability of these phenotype patterns with evidence for a single major gene and an autosomal dominant pattern of inheritance of the LDL phenotype B (Austin et al., 1986). Recently, the LDL phenotype B has been genetically linked to a locus on chromosome 19 near the LDL receptor gene locus (Nishina et al., 1992). Due to the complex interrelationship between VLDL, HDL and small dense LDL (Mc Namara et al., 1992), the increased risk of CHD associated with this subclass of LDL is not independent of its association with HDL and VLDL (Campos et al., 1992b). It has recently been demonstrated that small, dense LDL are more readily oxidised under various *in vitro* conditions (Tribble et al., 1992), probably because they carry less lipid-soluble antioxidants such as vitamin E. There is also evidence to suggest that small, dense LDL does not bind as well to LDL receptors as their larger counterparts (Kleinman et al., 1987a; Kleinman et al., 1987b). If this is also the case *in vivo*, generation of small, dense LDL will increase atherogenic potential.

1.5.4. High density lipoproteins

It has long been established that there is an inverse relationship between HDL and CHD (Miller and Miller, 1975). Subjects lacking Apo AI have been shown to lack HDL and some of these subjects have developed severe early atherosclerosis (Norum et al., 1982). Low HDL cholesterol levels are commonly associated with high concentrations of triglyceride-rich lipoproteins (Hulley et al., 1980). HDL is derived in part from surface components of VLDL, and therefore decreased HDL is consistent with accumulation of VLDL. Furthermore, elevated triglyceride levels can stimulate HDL cholesterol ester transfer, resulting in cholesterol ester depletion of HDL. It is believed that cholesterol ester transferred from HDL may then be delivered into the vessel wall as VLDL remnants or via their conversion to LDL, rather than being transported to the liver in HDL. This would explain the relationship between hypertriglyceridaemia, low HDL cholesterol levels and atherosclerosis.

Triglyceride-enrichment of HDL is also a consequence of cholesterol ester transfer with triglyceride-rich lipoproteins which can lead to alterations in the HDL particle size distribution. Patsch and co-workers (1984), proposed that excessive postprandial triglyceridaemia leads to cholesterol ester depletion of HDL_2. The triglycerides that replace the cholesterol ester are rapidly hydrolysed by HL producing smaller HDL_3 particles, a similar process to the production of small, dense LDL. A low HDL_2/HDL_3 cholesterol ratio is associated with atherosclerosis (Miller et al., 1981).

1.6. DIABETES MELLITUS

Diabetes Mellitus is a group of metabolic disorders characterised by chronic hyperglycaemia due to relative insulin deficiency and/or insulin resistance. Diabetes is present when, during a 75g oral glucose tolerance test, the fasting venous plasma glucose is more than 7.8mM or when the 2-hour value is more than 11.1mM. Two main types of diabetes exist:

1. Insulin-dependent diabetes mellitus (IDDM).

Patients with IDDM have absolute insulin lack. β-Islet cells of the pancreas fail to either synthesise or secrete insulin. IDDM is far less common than NIDDM, affecting only

10-20% of the diabetic population. β-cell deficiency is caused by destruction of the cell, and is probably due to genetic susceptibility, viral infection, and attack on the β-cells by autoantibodies. Because of their insulin deficiency, untreated IDDM always have elevated glucose levels, both because glucose fails to enter cells and is continually released by the liver. Lack of insulin stimulates glycogenolysis and proteolysis and elevates plasma levels of fatty acids and ketones due to increased lipolysis. Large amounts of glucose and ketones are often present in the urine. Patients with IDDM require insulin treatment to control symptoms and prevent ketoacidosis and death.

2. Non-insulin-dependent diabetes mellitus.

This type is the result of insulin resistance and relative insulin lack. Many patients with diabetes suffering from NIDDM can have near normal plasma glucose concentrations when fasting, and consequently have near normal insulin levels. In addition, NIDDM patients have enough insulin to suppress excess lipolysis, and to ensure adequate ketone entry into Kreb's cycle, thus avoiding ketoacidosis. They can often be adequately treated without insulin administration. However insulin's target cells do not respond normally due to alterations either in the insulin receptors or in the insulin receptor coupling mechanism.

Where hypocaloric dietary regulation and exercise are inadequate for reducing fasting hyperglycaemia, drugs such as sulphonylureas and metformin can be implemented. Sulphonylureas enhance the release of insulin from pancreatic β-cells. This restores acute insulin release in response to meals, which avoids the prolonged postprandial blood glucose elevation and subsequent hyperinsulinaemia. Metformin acts on insulin target cells resulting in enhanced insulin action. This reduction in insulin resistance results in an increase in peripheral glucose uptake and a decrease in hepatic glucose output.

Diabetic patients develop a variety of acute and chronic abnormalities which may be accompanied by lipid disorders and changes in lipid metabolism. Although most acute symptoms are linked to glucose metabolism, lipid handling is often deranged, with high levels of fatty acids, ketones and hypertriglyceridaemia. Chronic complications of diabetes may be specific to diabetes (microvascular disease; e.g. retinopathy, neuropathy and nephropathy) or reflect an increased risk of problems seen in the non-diabetic population (macrovascular disease; e.g. CHD). The two can be related because, for example, CHD is greatly increased in diabetic renal disease with proteinuria. Macrovascular disease is not only increased in diabetes, but also in normal individuals in whose blood glucose levels are in the top 5% of population values (Fuller et al., 1980). Furthermore, higher levels of blood

glucose and glycosylated haemoglobin (HbA1c) (as evidence of inadequate control), in non-diabetic subjects are associated with increased cardiovascular risk (Singer et al., 1992; Wilson et al., 1992). Other factors, in addition to glycaemic control, may also influence macrovascular disease. Hypertension, dyslipidaemia and hyperfibrinogenaemia are more likely to be present over many years, before and after diabetes diagnosis.

Diabetes is associated with a three- to four-fold increase in risk for CHD and vascular disease is the cause of three quarters of deaths (Garcia et al., 1974). Lipid and lipoprotein concentration abnormalities are common in diabetes but together with other risk factors does not account for the excess risk of CHD (Kannel and McGee, 1979). It is postulated that a range of quantitative changes in lipoprotein subfraction composition, not shown in basic lipid and lipoprotein levels, are present in diabetes and contribute to the excess risk of CHD.

1.6.1. Lipoprotein abnormalities in diabetes mellitus

Epidemiological studies have shown that hypertriglyceridaemia, but not hypercholesterolaemia, is the most common lipid abnormality in diabetic patients, the majority of whom have NIDDM (Barret-Connor et al., 1982; West et al., 1983; Wilson et al., 1985). In NIDDM patients the prevalence of hypertriglyceridaemia is 2-3 times higher than in the non-diabetic population (Barret-Connor et al., 1982).

1.6.1.1. Insulin-dependent diabetes mellitus

Triglycerides are elevated in poorly controlled IDDM (Bierman et al., 1966; Glasgow et al., 1981; James and Pometta, 1990) which may be due to increased rates of VLDL production in the liver (Nikkila et al., 1973). However, these levels are restored to normal in optimally-controlled patients (Nikkila et al., 1973). If poorly-controlled, HDL levels are low due to reduced LPL activity (Nikkila et al., 1977), but since insulin increases LPL activity, these levels are normalised in optimally controlled patients. Low LPL activity also results in diminished clearance of VLDL (Nikkila et al., 1977) resulting in accumulation of VLDL and chylomicrons, but this is reversed with insulin therapy. LDL is usually within normal limits, however, LDL production rates have been reported to be elevated in IDDM but return to normal after insulin infusion (Rosenstock et al., 1985). This may reflect the

increased synthesis of the precursor VLDL or the impaired removal of VLDL remnants, which are often measured as a component of LDL. In IDDM, LDL apoB has been shown to be glycosylated which may reduce receptor-mediated clearance of LDL (Kesaniemi et al., 1983; Uusitupa et al., 1986). Atherogenic VLDL and LDL subfraction profiles have been reported in IDDM (James and Pometta, 1990). Elevated plasma concentrations of the VLDL subfractions were observed, as well as a preponderance of small dense LDL compared to non-diabetic controls. Insulin treatment modified VLDL and LDL subfraction profiles so that they more closely resembled that of the controls.

1.6.1.2. Non-insulin-dependent diabetes mellitus

Triglyceride-rich lipoproteins

In NIDDM, elevated triglyceride is common (Howard, 1987) secondary to decreased removal and increased production (Kissebah et al., 1982), the latter of which is probably due to insulin resistance causing an increased flux of glucose and free fatty acids to the liver (Sniderman et al., 1993). The reduction in VLDL clearance may be due to insulin resistance impairing LPL activity (Pfeifer et al., 1983).

In obese Pima Indians with NIDDM, VLDL-triglyceride production tends to increase without an increase in VLDL-apo B suggesting an increase in particle size, while fractional catabolic rates of both VLDL-triglyceride and apo B are significantly lower than in those without diabetes (Taskinen et al., 1986). Sulphonylurea therapy can improve these abnormalities: LPL activity increased and hepatic lipase activity decreased with treatment and the VLDL particle size (triglyceride : apo B ratio) returned to normal (Taskinen et al., 1986).

Large, triglyceride-rich VLDL may, like chylomicron remnants, be removed by apo E recognition. In NIDDM, this clearance is normal or impaired in the case of hypertriglyceridaemia (Nikkila et al., 1977; Kissebah et al., 1982) resulting in accumulation of VLDL remnants, which are known to be atherogenic (Goldstein et al., 1980, Mahley et al., 1984). In NIDDM, VLDL apolipoprotein composition is abnormal with a decreased proportion of apo C to apo E which may influence metabolism by macrophages (Klein et al., 1990) . In addition, VLDL apo E may be glycosylated and an increase in the more acidic and sialated forms of apo E have been found in diabetes (Black et al., 1990) which may affect binding to the B/E receptor.

Considerable heterogeneity of triglyceride-rich lipoproteins with structural and functional diversity has been demonstrated in the non-diabetic population (Packard et al., 1979; Packard et al., 1984). More recently (Taskinen et al., 1990), it was reported that patients with NIDDM had an abnormal VLDL apo B metabolism. It was demonstrated that patients with NIDDM preferentially produce larger VLDL particles, which, upon insulin treatment, were reduced in favour of an increase in smaller VLDL particles. This change led to a normalisation of apo B kinetics, since small VLDL was more readily converted to LDL than large VLDL. The fractional catabolic rate of LDL increased and LDL production in the liver was reduced. In NIDDM with poor control and mild hypertriglyceridaemia, both VLDL and LDL subfraction profiles were grossly abnormal and potentially atherogenic (James and Pometta, 1991). Larger cholesterol ester-rich VLDL was more prevalent than the small triglyceride-rich VLDL. Substantial but incomplete improvement occurred with better glycaemic control. Similarly, in patients with NIDDM, improved diabetic control achieved with insulin infusions from a programmed implanted pump, corrected triglyceride-rich lipoprotein subfraction profiles towards that of non-diabetics (Georgopoulos et al., 1992). In another study however, Patti and co-workers (1991) reported an increase in the smallest cholesterol-enriched VLDL subfraction in normotriglyceridaemic patients with NIDDM compared to non-diabetic controls.

Until recently, most of the investigations into lipaemia in NIDDM have been performed in the fasting state. However, triglyceride-rich lipoprotein abnormalities and clearance defects may be more evident when examined in the postprandial state. A recent comparison of postprandial lipemia in NIDDM with hypertriglyceridaemia compared to NIDDM with normotriglyceridaemia, showed lipaemia was due to high levels of chylomicron remnants. These subjects had higher concentrations of small dense LDL which correlated with fasting triglyceride and extent of postprandial lipemia (Tan et al., 1992). Also it has been demonstrated that normotriglyceridaemic patients with NIDDM have an excess of chylomicron remnants after an oral fat load (Chen et al., 1993), suggesting that the degree of postprandial lipaemia is greater in patients with NIDDM. Diabetics with fasting chylomicronaemia develop monocyte-macrophage derived foam cells in eruptive xanthomas that are filled with triglyceride and cholesteryl ester (Parker et al.,1970). Insulin normalises this leaving only cholesterol ester in the foam cells. The cause of delayed clearance of chylomicron remnants remains to be determined, however low LPL activity, commonly found in NIDDM, may play a role.

Low density lipoproteins

The plasma concentrations of LDL cholesterol are usually found to be within normal limits in NIDDM patients (Barrett-Connor et al., 1982). However, recently levels of LDL have been found to be raised in patients with NIDDM (Harris, 1991). This may be due to down-regulation of LDL receptors on hepatocytes caused by insulin resistance (Chait et al., 1979). In patients with marked hypertriglyceridaemia, LDL removal is impaired (Kissebah et al., 1983). Triglyceride-enriched LDL has decreased ability to bind to fibroblasts and internalise cholesterol and therefore cannot downregulate cholesterol synthesis (Hiramatsu et al., 1985). On the other hand, large VLDL, common in diabetes (particularly with hypertriglyceridaemia), is less readily converted to LDL (Stalenhoef et al., 1984, Packard et al., 1984) which may balance out the aforementioned reasons for LDL accumulation.

Cumulative flotation ultracentrifugation has been used to show that small dense LDL (LDL-3) is particularly common in poorly-controlled, hypertriglyceridaemic patients with NIDDM (James and Pometta, 1991). Concentrations of LDL-3 were reduced after insulin therapy. Feingold and co-workers (1992) reported that phenotype B was more common in normolipidaemic NIDDM than in normolipidaemic controls. Phenotype B was associated with an increase in plasma triglyceride and low HDL-cholesterol. This association was not seen in NIDDM patients with type A phenotype. Although small dense LDL has been associated with CHD in the general population (Austin et al., 1988), no studies of this association have been reported in diabetics. Tilley-Kiesi (1992) investigated the LDL profile in normolipidaemic NIDDM (+\- CHD) and in controls (+\- CHD). It was reported that NIDDM and controls with CHD had larger LDL than NIDDM and controls without CHD due to a higher triglyceride content. However, in the non-diabetic population, small LDL is more prevalent in men with CHD than in men without CHD (Campos et al., 1992b), and small LDL was associated with high plasma triglyceride and low HDL-cholesterol (Campos et al., 1992b; Mc Namara et al., 1992). These authors concluded that LDL size is not an independent discriminator for CHD after conventional risk factors such as LDL-cholesterol and HDL-cholesterol have been taken into account.

It would appear that small dense LDL found in patients with NIDDM might be the consequence of high levels of VLDL, particularly large, triglyceride-rich VLDL in these patients. Small, dense LDL are derived from large triglyceride-rich VLDL in the hypertriglyceridaemic state, whereas in the normotriglyceridaemic state, the liver produces small VLDL particles which give rise to larger, more buoyant LDL (Caslake et al., 1992). In addition, patients with NIDDM have been shown to have increased CETP (Bagdade et

al., 1993) and HL activities (Harno et al., 1980). Furthermore, LDL size and peak density have been shown to be related to insulin resistance in NIDDM (Stewart et al., 1993), and nondiabetic subjects with LDL subclass phenotype B are more insulin-resistant than those with subclass phenotype A (Reaven et al., 1993; Selby et al., 1993). The mechanism(s) explaining the link between insulin resistance and LDL subfractions remain to be determined, but it is also known that insulin resistance is associated with increased production of large triglyceride-rich VLDL (Taskinen et al., 1990) which may give rise to small, dense LDL.

An additional abnormality of LDL in NIDDM is glycation (Kim et al., 1982). The extent of glycation of LDL in patients with NIDDM with moderate hypertriglyceridaemia is 2-5% and has been shown to decrease LDL metabolism by 5-25% (Steinbrecher et al., 1984). Glycation may not only promote foam cell formation, but may also confer increased susceptibility of the LDL in individuals with diabetes to oxidation (Hunt et al., 1990). In addition, small, dense LDL, commonly found in NIDDM, is more prone to oxidation thus further increasing the atherogenic potential of diabetic LDL.

High density lipoproteins

HDL cholesterol levels are frequently found to be reduced in patients with NIDDM (Lopes-Virella et al., 1977; Barrett-Connor et al., 1983; Howard, 1987;). This is consistent with impaired LPL activity (Pfeifer et al., 1983), increased HL activity (Harno et al., 1980) and decreased VLDL clearance (Howard, 1987) in these patients. Insulin resistance is associated with low HDL levels (Laakso et al., 1990). Intensive insulin therapy in NIDDM patients is associated with an increase in HDL_2 which is positively correlated with LPL activity during insulin therapy (Taskinen et al., 1988). Triglyceride-enrichment of HDL is common and may occur as the result of cholesterol ester transfer with triglyceride-rich lipoproteins (Tall, 1986).

1.6.2. Treatment of lipid disorders and relevance to diabetes

Lipid-lowering therapy has been shown to reduce progression of coronary lesions (Levy et al., 1984) and increase regression in coronary lesions (Blankenhorn et al, 1987). Prognosis for individuals with pre-existing CHD can be improved by lipid-lowering therapy (Carlson et al., 1988; Blankenhorn et al., 1987; Brown et al., 1990; Kane et al., 1990). Recently, metanalysis has demonstrated from cohort studies of half a million men that a long term reduction in serum cholesterol of 10% lowers the risk of CHD by 50% at age 40 and by 20% at age 70 (Law et al., 1994). Although there are no comparable studies in diabetes, there is no reason to believe that similar therapy would be less beneficial in diabetic compared to non-diabetic subjects.

Hypertriglyceridaemia has been shown to be a significant predictor of subsequent cardiovascular mortality in diabetes (Fontbonne et al., 1989) and the Stockholm study (Carlson et al, 1988) demonstrated that a reduction in triglyceride levels can be an important factor in decreasing cardiovascular mortality. Where diabetic dyslipidaemia does not respond to diet, exercise, lifestyle and glycaemic control, lipid-lowering drugs should be considered. Fibrate drugs are often first choice, but other drug groups such as HMGCoA reductase inhibitors, may be needed. The fibric acid group of drugs have been associated with significant reductions in coronary events in the Helsinki Heart Study, especially in patients with combined hyperlipidaemia (Manninen et al., 1987).

1.6.2.1. Fibric acid derivatives

Fibric acid derivatives (eg. gemfibrozil, ciprofibrate, fenofibrate and bezafibrate) have the ability to lower plasma and VLDL triglyceride by increasing LPL activity, reducing fatty acid release from adipose tissue and inhibiting hepatic triglyceride synthesis, and are therefore beneficial for treating dyslipidaemia in NIDDM. Several investigations have shown that fibrates affect VLDL composition and heterogeneity (Eisenberg et al., 1984; Paucillo et al., 1990; Winocour et al., 1992). Paucillo et al. (1990) reported that in hypertriglyceridaemic patients, gemfibrozil reduced the levels of the larger VLDL subfractions, while the levels of the smallest fractions remained the same. Fibrates may increase LDL cholesterol levels in hypertriglyceridaemic subjects (Carlson et al., 1977), because normalising triglyceride-rich lipoprotein metabolism allows conversion of VLDL to LDL, rather than conversion to IDL which is removed by the liver. In addition, normalising

triglyceride-rich lipoprotein metabolism results in restoration of cholesterol ester-rich LDL which previously was triglyceride enriched due to cholesterol ester transfer. This increase in LDL cholesterol is not seen in hypercholesterolaemic subjects after fibrate therapy. Increased receptor mediated catabolism of LDL (Series et al., 1989, Stewart et al., 1982) has been demonstrated *in vivo* after bezafibrate therapy. This may be caused by increased receptor expression mediated by enhanced secretion of cholesterol into the bile, known to occur after fibrate therapy. Alternatively, changes in LDL composition may provoke more avid binding to the receptors.

Fibrates also affect LDL density. It appears that fibrate therapy can reduce levels of small dense LDL (Eisenberg et al., 1986). Small dense LDL subspecies have been changed to larger less atherogenic LDL by gemfibrozil in type II hyperlipidaemic patients (Tsai et al., 1992). A reduction in small dense LDL after fibrate therapy is in agreement with a reduction in plasma triglyceride.

Fibrates increase HDL cholesterol levels (Rouffy et al., 1985; Sorisky et al., 1987) and this is likely to be due to increased VLDL catabolism. In addition, HDL cholesterol will increase as the amount of triglyceride-rich lipoproteins available for cholesterol ester transfer is reduced.

Postprandial lipemia in hypertriglyceridaemic subjects is reduced by fibrates which is probably due to the increase in catabolism of chylomicrons and VLDL (Oster et al., 1985; Weintraub et al., 1987; Simpson et al., 1990). Fibrates such as gemfibrozil have been shown to increase HDL_2 and HDL_3 in the postprandial state in hypertriglyceridaemic subjects (Ditschuneit et al., 1992).

Bezafibrate, has similar effects on lipoprotein metabolism as previously described for gemfibrozil and other fibrates. Bezafibrate has been reported in NIDDM to reduce serum triglyceride and cholesterol as well as increase HDL (Seviour et al., 1988; Rovellini et al., 1992). In patients with IDDM and hypercholesterolaemia, bezafibrate can reduce cholesterol and triglyceride of large VLDL and reduce free cholesterol and esterified cholesterol in IDL (Winocour et al.,1992). In the same study, in patients with IDDM and hyperlipidaemia, bezafibrate again reduced large VLDL cholesterol and triglyceride, but reduced free and esterified cholesterol in small VLDL. In both cases LDL composition was unchanged. Small, dense LDL subspecies have been changed to larger less atherogenic LDL by bezafibrate in patients with hyperlipoproteinaemia (Homma et al., 1994).

Reductions in fasting blood glucose after bezafibrate therapy have been reported in patients with NIDDM (Seviour et al., 1988; Jones and Alberti., 1988; Mikhailidis et al.,

1990; Rovellini et al., 1992), although Riccardi and co-workers (1989) found no change in glucose levels. In addition, bezafibrate therapy has been reported to reduce non-esterified fatty acids (NEFA) concentrations in NIDDM (Alberti et al., 1990; Jones et al., 1990).

1.6.2.2. HMG-CoA reductase inhibitors

Lovastatin, simvastatin, fluvastatin and pravastatin are competitive inhibitors of HMGCoA reductase, the rate-limiting enzyme of cholesterol synthesis. These drugs are the most potent available for reducing plasma concentrations of LDL cholesterol. Their inhibition of HMGCoA reductase, and thus cholesterol synthesis, results in the up-regulation of LDL receptor expression on the cell surface enhancing uptake of LDL and VLDL remnants from the plasma. Concurrently, hepatic synthesis of VLDL and LDL may be reduced. They also produce moderate increases in HDL.

Recently, the effect of these drugs in patients with NIDDM have been investigated. Lovastatin has been shown to reduce LDL and VLDL cholesterol levels and increase HDL cholesterol levels in NIDDM patients with primary hypercholesterolaemia (Golberg et al., 1990). Studies have shown statins to be better at reducing LDL cholesterol levels and fibrates better at reducing triglyceride levels and increasing HDL levels (Crepaldi et al., 1990; Ojala et al., 1990). Fibrates have also been shown to be better than statins at reducing postprandial lipaemia (Simo et al., 1993). In hypertriglyceridaemic subjects gemfibrozil reduced chylomicron triglyceride and there was a close correlation between change in fasting triglyceride and change in postprandial response in subjects taking gemfibrozil but not in those taking lovastatin. Also LPL and HL activities were increased by gemfibrozil but not by lovastatin.

1.7. SYSTEMIC LUPUS ERYTHEMATOSUS AND PSORIATIC ARTHRITIS

Systemic lupus erythematosus (SLE) is an autoimmune disease caused by circulating immune complexes and is characterised by anti-nuclear factor and other auto-antibodies. The incidence of SLE is highest in people of 20-40 years and is more common in females than in males. Active SLE symptoms such as inflammation of joints usually occur at the onset of the disease and therapy is required, whereas inactive SLE is asymptomatic and does not usually require therapy. SLE has a bimodal pattern of morbidity and mortality (Urowitz et al., 1976). A major contribution to this pattern is made early in the disease by renal and cerebral involvement. It is now recognised that the late morbidity and mortality is related to accelerated atherosclerosis. Many factors contribute to hyperlipidaemia in patients with SLE, such as a persistent nephrotic state (Appel et al., 1985) and use of corticosteroids (Ettinger et al., 1987) the latter of which causes elevated plasma levels of triglyceride, LDL cholesterol and apo B100 (Ettinger and Hazzard, 1988). However active SLE itself contributes to a distinct pattern of dyslipidaemia (Ilowite et al., 1988) with raised serum and VLDL triglyceride, and reduced HDL cholesterol. On the other hand, one study (Leong et al., 1994) found no such activity related differences.

Certain cytokines expressed by macrophages (interleukin-1 and tumour necrosis factor-α) are known to suppress LPL activity (Beutler et al., 1985; Fried and Zechner, 1989; Ogawa et al., 1989) and to increase oxidative metabolism, having atherogenic implications. These cytokines are upregulated in SLE.

Hydroxychloroquine is used for treating SLE patients with a milder form of the disease. It has potential lipid-lowering properties and has been shown to reverse deleterious effects of steroids on lipids separate to its steroid-sparing activity in patients with rheumatic disease (Wallace et al., 1990). Recently, hydroxychloroquine has been associated with reduced plasma levels of triglyceride, VLDL triglyceride, LDL triglyceride, HDL triglyceride and VLDL cholesterol in SLE (Hodis et al., 1993), and therefore may be useful in ameliorating the effects of the active disease as well as effects of steroid therapy on triglyceride-rich lipoprotein metabolism.

Psoriasis is a skin disease characterised by the appearance of scaly patches on the surface of the skin. The onset of the disease usually occurs between the ages of 20 and 50 years, and is as common in males as it is in females. Ten percent of patients develop psoriatic arthritis affecting many joints, particularly small joints of the hand. Recently, dyslipoproteinaemia has been reported in subjects with rheumatoid arthritis (RA) and

psoriatic arthritis (PA) (Lazarevic et al., 1992). Both patient groups showed a reduction in serum cholesterol, LDL cholesterol and HDL cholesterol compared to normal healthy controls. There were no differences in plasma triglycerides. These changes were ameliorated in severe cases of the disease, with the additional lowering of serum triglycerides in PA patients, which is in agreement with the findings of Jones et al. (1993). As disease activity decreased with treatment, RA patients showed normalisation of almost all serum lipid concentrations. Similar findings were reported in RA patients from two parallel studies (Svenson et al., 1987a; Svenson et al., 1987b). Because the severity of the disease is parallel to the degree of dyslipidaemia, the level of inflammation activity may play a role in the development of dyslipidaemia in RA and PA.

1.8. XANTHINE OXIDASE

1.8.1. PROPERTIES OF XANTHINE OXIDASE

Xanthine oxidase is an enzyme which has the unusual property that it can exist in a dehydrogenase form (D-type) which uses NAD^+ as an electron acceptor and an oxidase form (O-type), which uses oxygen as an electron acceptor. The enzyme was one of the first flavoproteins to be purified (Ball, 1939) and it was later found to contain molybdenum (De Renzo et al., 1956) and iron (Rithert and Westerfeld, 1954). The flavin molecule together with these metals plays an important role in the enzyme's catalysing capacity. Xanthine oxidase is a dimer containing two subunits of 150KD; each subunit contains as cofactors involved in catalysis one molybdenum molecule, two iron-sulphur centres and one FAD group. The reducing substrate (e.g. purines, aldehydes, pteridines) is oxidised at the molybdenum site and electrons that become available are channelled via the iron-sulphur centre to FAD where the oxidising substrate (e.g. NAD^+, O_2, methylene blue) is reduced.

Xanthine oxidase (D-type) has the physiological function in purine catabolism of oxidising hypoxanthine to xanthine and then to uric acid (urate), using NAD^+ as an electron acceptor.

$$\text{Hypoxanthine} + H_2O + NAD^+ \rightarrow \text{Xanthine} + NADH + H^+$$

$$\text{Xanthine} + H_2O + NAD^+ \rightarrow \text{Uric acid} + NADH + H^+$$

The enzyme also has the unusual property that when molecular oxygen serves as the electron acceptor for that reaction, oxygen is reduced to superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2).

$$\text{Hypoxanthine} + 2H_2O + 2O_2 \rightarrow \text{Xanthine} + O_2^{\cdot-} + H_2O_2$$

$$\text{Xanthine} + 2H_2O + O_2 \rightarrow \text{Urate} + O_2^{\cdot-} + H_2O_2$$

Hydrogen peroxide, itself is a moderately strong oxidant, can react with a superoxide radical and through the Haber-Weiss reaction produce the very toxic hydroxyl radical (Haber and Weiss, 1935; McCord et al., 1978). Therefore the enzyme has the capacity to produce essentially toxic, reactive oxygen species (ROS). Xanthine oxidase and ROS produced by the enzyme have been proposed to play a role in a number of pathological conditions, some of which include cerebral ischaemia (Kontos et al.,1981), irradiation damage (Petkav et al.,1978), intestinal ischaemia (Granger et al.,1981), pulmonary disorders (Johnson et al., 1981), autoimmune rheumatic diseases (Miesel et al., 1993), atherosclerosis (Ohara et al.,1993), and gout (Kelley et al.,1989).

Xanthine oxidase exists *in vivo* predominantly in the dehydrogenase form, which can be converted to the oxidase form by a number of processes including proteolysis, heating, storage at -20°C, anaerobiosis, certain organic solvents, incubation with subcellular fractions and sulphydryl (SH) reagents (Stirpe and Della Corte, 1969).

Dithiothreitol, a SH-reductant, can prevent conversion of the D-type to the O-type and even reverse the conversion induced by different treatments (Della Corte and Stirpe, 1972), except for proteolysis, which is irreversible. Therefore the conversion from the D-type to the O-type could occur reversibly by oxidation of sulphydryl groups or irreversibly by proteolytic cleavage. Exceptionally, the conversion of the O-type to the D-type by SH-reductants was not possible in the intestine (Battelli et al., 1972), probably because of the high proteolytic activity derived from the pancreatic enzymes. On the whole, protease inhibitors are adequate in preventing proteolytic conversion. Isolation of the two distinct forms has permitted characterisation of these enzyme forms.

The D-type and O-type differ in their protein environment around the flavin group, which is possibly responsible for the different reactivities toward NAD^+ and O_2 (Massey et al., 1989; Saito et al., 1989). Recently, it has been suggested that the molybdenum centre of the two enzyme types is different (Hunt et al., 1993). NADH and NAD^+ are used as reducing and oxidising agents respectively at a high rate by the D-type (Rajagopalan and

Handler, 1967; Schopfer et al., 1988) whereas they only react slowly or not at all with the O-type (Massey et al., 1969). On the other hand, the D-type but not the O-type reacts only slowly with atmospheric oxygen in the presence of NAD^+. Despite the classical role of the D-type in using NAD^+ as an electron acceptor and the O-type in using O_2 as an electron acceptor, the D-type, in the absence of NAD^+ has been reported to produce 9-15% of urate compared with the O-type in aerobic conditions, with concomitant production of ROS (Saito and Nishino, 1989; Hunt and Massey, 1992). Clearly the rate of O_2 consumption by the D-type is lower than the O-type in the absence of NAD^+, but it is not negligible.

The presence of inactive demolybdo- and desulpho-enzyme molecules have been known for a long time (Massey and Edmonson, 1970; Johnson et al., 1974; Bray, 1975; Harrison et al., 1991). In bovine milk, 20-40% desulpho-enzyme molecules were found (Massey et al., 1969; Bray, 1975). Abadeh and co-workers (1992a) found that in human milk, more than 98% of total xanthine oxidase was in an inactive form with approximately 26% in the demolybdo-form and the remainder in the desulpho-form. It has been suggested that interconversion of inactive to active form was regulated posttranslationally by incorporation or subtraction of an essential sulphur atom at the molybdenum centre (Coughlan et al., 1981).

1.8.2. Distribution of xanthine oxidase

Xanthine oxidase is widely distributed in the tissues of many species, including bacteria (Bray, 1988) and plants (Perez-Vicente et al., 1992). A variety of biochemical methods have been used to detect xanthine oxidase in mammalian tissues (Parks and Granger, 1986), including most tissues in rat (Hashimoto, 1974; Parks and Granger, 1986).

In humans, high activity has only been detected in the liver and small intestine (Watts et al., 1965; Brunschede and Krooth, 1973), whereas low activity was found in other organs, particularly heart (Eddy et al., 1987; Muxfelt et al., 1987; Grum et al., 1989; De Jong et al., 1990; Kooij et al., 1992a; Abadeh et al., 1992b; Abadeh et al 1993). However, two studies (Krenitsky et al., 1974; Wajner and Harkness, 1989) found high activity in many human tissues. These contradictions could be explained by assumption that tissues were sampled from patients with intestinal and liver disease and therefore may have high xanthine oxidase activity in the blood (De Jong et al., 1990; Kooij et al., 1992a).

Biochemical methods do not discriminate, however, between the activity of the enzyme in different cell types within one tissue, and accordingly histological methods have

been developed. These techniques permit localisation of xanthine oxidase enzyme activity by the reduction of nitro-blue tetrazolium to insoluble formazan in the presence of hypoxanthine. Enzyme activity has been localised in cell epithelium of rat duodenum (Pricket et al.,1970), in the cytoplasm of rat (Auscher et al.,1977) and hamster hepatocytes (Ibrahim and Stoward, 1978), and in the renal collecting tubule cells of hamsters (Ibrahim and Stoward, 1978). Recently, xanthine oxidase activity has been demonstrated in epithelium, endothelial cells and fibroblasts of all organs of rat except brain (Kooij et al.,1992b), and only in the small intestine and liver in humans (Kooij et al., 1992a).

Immunohistochemical techniques have been used antibodies to detect xanthine oxidase (Jarasch et al.,1981) in the cytoplasm of capillary endothelial cells of bovine mammary glands, liver, heart and intestine, but not in larger blood vessels. Later, in humans, activity was detected in the endothelial cells of capillaries of heart, liver, placenta and kidney (Jarasch et al.,1986; Bruder et al.,1984).

This discovery of xanthine oxidase protein in the endothelial cells of heart and tissues other than liver and intestine is in contrast to many biochemical and histochemical findings. However, the former results have recently been supported by a number of studies (Abadeh et al., 1993; Hellsten-Westing, 1993; Moriwaki et al.,1993). Abadeh and co-workers (1993) found similar amounts of enzyme molecule in human and rat heart by immunoprecipitation, but activity was 100-fold higher in rat. It was suggested that this lack of activity was due to the human enzyme being in the desulpho- and demolybdo-form. Therefore it would seem that enzyme is present in these tissues, but its activity is below the detection limits of most methods. However it is possible that enzyme from human intestine and liver is released into the blood after injury, and sticks to the endothelium of capillaries of other tissues, since capillaries of small vessels, but not of large can endocytose macromolecules (Jarasch et al., 1981). This is consistent with the presence of antibodies to xanthine oxidase in human blood (Bruder et al., 1984; Harrison et al., 1990; Ng et al., 1990) and that the presence of xanthine oxidase but not activity has been detected in endothelial cells of human capillaries other than liver and intestine.

1.8.3. Function of xanthine oxidase

Xanthine oxidase, in humans is involved in purine catabolism by catabolising hypoxanthine to xanthine and then to uric acid. However the contribution of xanthine oxidase in purine catabolism is only minor because of the well-developed salvage pathway.

In bacteria, its main function is oxidation of purines to provide carbon, nitrogen and energy (Wagner et al., 1984). Xanthine oxidase is found exclusively as the non-convertible D-type in all organisms except mammals, and therfore xanthine oxidase might have functions in mammals specific to ROS. It has been proposed (Kooij et al., 1992b), because enzyme activity was found only in the small intestine and liver in humans, that in the intestine ROS from the O-type enzyme are involved in proliferation and/or differentiation of epithelial cells when they migrate to the apical villus.

With respect to a possible antioxidant function in humans, it is possible that the enzyme participates in the antioxidant defence system against radicals produced in the liver and/or blood, since urate, the product of the enzyme, has been found in high concentrations in blood and is a strong scavenger of free radicals (Ames et al., 1981). These authors suggested that this may contribute to the larger life span of humans compared to other mammals. On the other hand, Koster and Slee (1983) did not find any free radical scavenging properties of urate. More recently, urate has been suggested to play a role in preventing generation of lipid peroxidation produced during mastication and digestion of ingested foods (Terao and Nagao, 1991) and as an antioxidant in human nasal mucosa (Peden et al., 1990).

Since H_2O_2 and $O_2^{\cdot-}$ are produced in virtually all reactions catalysed by O-type xanthine oxidase, the enzyme also serves as a ubiquitous source of oxidising agents. Indeed, xanthine oxidase has been proposed as a source of oxygen radicals in polymorphonuclear leukocytes (Tubaro et al., 1980), and in this way the enzyme may have a protective role against bacteria.

1.8.4. Ischaemia-reperfusion with special reference to the heart

Involvement of xanthine oxidase in the induction of tissue damage during reperfusion after ischaemia was first indicated when it was found that allopurinol, an inhibitor of xanthine oxidase, prevented the rise of urate levels in the blood and increased survival rate of dogs after haemorrhagic shock (Crowell et al.1967; Jones et al.,1968). Saugstadt and Aasen (1980), showed that hypoxanthine levels in the blood during hypoxia were inversely related to survival rate of pigs. They suggested that superoxide anion radicals are produced during reperfusion of xanthine oxidase using the increased amounts of hypoxanthine that becomes available during ischaemia.

Granger and co-workers (1981), demonstrated oxygen free radicals could be involved in ischemia reperfusion injury because a marked decrease in injury was observed in intestinal tissue upon administration of superoxide dismutase or catalase, scavengers of oxygen-derived radicals and hydrogen peroxide respectively. They proposed that the dehydrogenase form of xanthine oxidase is converted to the oxidase form during ischaemia and ATP is broken down into hypoxanthine. Upon reperfusion, oxygen is introduced and xanthine oxidase consumes hypoxanthine with the production of ROS which causes tissue injury. Over the past decade, XO involvement in tissue injury due to ischaemia reperfusion process has been postulated in a number of tissues, some of which are shown below in Table 1.2.

Table 1.2. Tissues in which the involvement of XO in injury due to ischaemia reperfusion process has been postulated.

Tissue	Reference
Heart	McCord et al., 1985; Brown et al., 1988; Terada et al., 1991
Liver	Schneider et al., 1991; Suematsu et al., 1992
Lung	Ishi et al., 1992; Repine et al., 1992; Ward, 1991
Intestine	Granger et al., 1981; Granger et al., 1988; Rangan and Buckley, 1993
Skeletal Muscle	Suzuki and Ford, 1991; Punch et al., 1992
Eye	Peachey et al., 1993
Skin	Im et al., 1989; Pokorny et al., 1989
Brain	Lin and Phillis, 1991; Thom, 1992
Kidney	Schiller et al., 1991

The conversion of D-type xanthine oxidase to O-type has been demonstrated in regional myocardial ischaemia (McCord, 1985; Chambers et al.,1985). McCord (1985) postulated that ROS generated by xanthine oxidase could cause cellular damage of myocardial tissue during reperfusion leading to myocardial infarction. However the effects of xanthine oxidase inhibitors on experimental myocardial infarction have been variable.

Treatment with allopurinol one day before coronary artery occlusion followed by reperfusion limited the extent of canine myocardial infarction (Werns et al.,1986; Charlat et al.,1987). However, when treatment was administered only 30 mins before coronary artery occlusion allopurinol did not reduce extent of myocardial injury (Reimer et al.,1985).

This failure of acute treatment to reduce myocardial injury suggests that allopurinol requires longer treatment time to allow conversion of allopurinol to oxypurinol, its active metabolite, which is a non-competitive inhibitor with a longer half-life (Spector et al.,1988). Also short term administration of the drug may merely delay tissue injury, while substantial therapy may be required to prevent infarction. Low doses may actually increase reperfusion injury because a reduction in urate concentrations may lead to a reduction in free radical scavenging. Zhong and co-workers (1989) reported that, in liver tissue, cell injury was increased with low doses of allopurinol while high doses prevented cell death.

Furthermore, it is possible that myocardial protection due to allopurinol is not due to its inhibition of xanthine oxidase. Allopurinol was reported to limit infarct size in rabbit and pig although xanthine oxidase activity was not detected in myocardium of either species (Godin and Bhimji, 1987; Das et al., 1987). Also amflutizole, another potent inhibitor of xanthine oxidase, did not reduce infarct size in the dog (Werns et al., 1989). *In vitro* data has demonstrated that oxypurinol acts as a scavenger for hydroxyl radicals (Hoey et al., 1988), and perhaps it is via free radical scavenging that allopurinol reduces infarct size.

The conversion of D-type xanthine oxidase to O-type in ischaemia regarding the production of ROS during reperfusion is still a controversial issue (Brass et al.,1991; Battelli et al.,1992). Furthermore, it has been demonstrated that xanthine oxidase in the dehydrogenase form is capable of producing ROS in the presence of oxygen and absence of NAD^+ (Saito and Nishino, 1989; Hunt and Massey, 1992) suggesting that conversion from the D-type to the O-type is not a requirement for ischemia-reperfusion injury.

Most biochemical assays could not detect xanthine oxidase activity in human tissues, except liver and intestine (Eddy et al., 1987; Muxfelt et al., 1987; Grum et al., 1989; De Jong et al., 1990, Kooij et al., 1992b; Abadeh et al., 1992b; Abadeh et al., 1993), which argues against a major role for xanthine oxidase in most organs. On the other hand, these studies cannot exclude the possibility that the enzyme is present in an inactive form. Immunohistochemical studies have detected xanthine oxidase in human heart (Bruder et al., 1984; Jarasch et al., 1986; Abadeh et al., 1993), and Abadeh et al., (1992a) reported that xanthine oxidase from human milk was 98% inactive, 26% of which was in the demolybdo-form and the remainder was in the desulpho-form. It was reported that activity was 100 fold less in human heart than in rat heart (Abadeh et al.,1992b; Abadeh et al., 1993). This would suggest that the presence of xanthine oxidase in human heart occurs predominantly in the inactive form as shown for the enzyme in human milk (Abadeh et al., 1992a). This would account for the low or zero activity of xanthine oxidase often reported in human heart, and for the ability of Jarasch and co-workers (1986) to locate the enzyme in human

heart. It is interesting that the inactive form of human milk enzyme, while incapable of oxidising most human substrates, can, nevertheless, oxidise NADH generating free radicals (Nakamuru, 1991; Abadeh et al., 1992a). The presence of a similar enzyme in human heart may well contribute to ischaemia-reperfusion injury in this tissue.

1.8.5. Anti-xanthine oxidase antibodies and heart disease

Studies by Davies and colleagues (Davies et al., 1969; Davies et al., 1974) reported that antibodies to cows' milk, which are present in most subjects, were higher in patients who had suffered a myocardial infarction than in controls, findings that were confirmed by one study (Oster et al., 1974), but not supported in others (Toivanen, 1975; Scott, 1976). These studies measured antibodies to dried milk powder, clearly a complex antigenic mixture. However, Davies (1980) found that the antibodies to dried whole milk, as detected by haemagglutination assay, were largely directed toward the washed cream fraction, and later reported (Davies et al., 1982) that the antibodies were directed to bovine milk fat globule membrane.

Harrison et al. (1990) pursued this and showed that human antibodies against bovine milk fat globule membrane themselves interact primarily with the enzyme xanthine oxidase. They went on to develop an enzyme linked immunosorbent assay (ELISA) for anti-xanthine oxidase antibodies and compared their levels in the serum of 107 patients who had suffered a myocardial infarction with those in 86 controls Levels of IgM anti-XO antibodies were significantly higher in the patients with myocardial infarction. There were no differences found for IgA and IgG anti-XO antibodies, and total levels of IgM did not differ between patients and controls. Serial assays following myocardial infarction showed no evidence that raised levels of IgM anti-XO antibodies resulted from the infarction itself.

While all human sera tested, whether from normal or diseased donors, have been shown to contain anti-XO antibodies (Harrison et al., 1990), their origin and role are far from established. They could arise in response to dietary cow's milk. Uptake of macromolecules into the bloodstream from the intestine has been reported in humans (Walker et al., 1974) and moreover, it has been claimed that human anti-XO antibody levels depend on dairy food consumption (Rzucildo et al., 1979; Deeth, 1983). Alternatively these antibodies could arise in response endogenous enzyme which is known to be present in the capillary endothelium of many tissues (Jarasch et al., 1981; Jarasch et al., 1986).

If the antibodies are in fact autoantibodies, it is tempting to cast them in a protective role in the elimination from the circulation, of xanthine oxidase, with its potential for generation of destructive ROS.

1.9. AIMS OF THE THESIS

1. Lipid and lipoprotein abnormalities, together with other risk factors, do not account for the excess risk of CHD associated with NIDDM. It is postulated that changes in fasting and postprandial lipoprotein quantity and quality, not shown by basic lipid and lipoprotein levels, are present in NIDDM and contribute to the excess risk of CHD. In view of this, fasting and postprandial lipoprotein subfraction distribution and composition were studied in patients with NIDDM, firstly in comparison with non-diabetic controls (Chapter 3) and secondly as part of fibrate intervention trial (Chapter 4).

Dyslipoproteinaemia has been reported in patients with SLE and psoriatic arthritis. To investigate the lipoprotein profile of these patients in more detail, lipoprotein subfraction distribution and composition were examined (Chapter 5).

2. Studies have indicated that xanthine oxidase could play an important role in myocardial ischemia reperfusion injury. More recently, it has been reported that antibodies to xanthine oxidase, present in the serum of all human subjects, were raised in patients with myocardial infarction. It is clearly important to study the occurrence and pathological significance of anti-XO antibodies in human serum. To this end, levels of antibodies were compared in serum from normal healthy donors of varying age and gender (Chapter 6), in patients with myocardial infarction (Chapter 7) and in patients with a high risk of CHD (patients with diabetes) (Chapter 8).

CHAPTER 2

GENERAL METHODS

2.1. REAGENTS

Apo AI and apo B kits were supplied by Sigma (St. Louis, Missouri, USA).
Cholesterol, triglyceride, phospholipid and free cholesterol kits were supplied by Boehringer Mannheim (Lewes, East Sussex, U.K.).
Insulin kits were supplied by Incstar Corporation (Stillwater, Minnesota, USA).
Glucose kits were supplied by Randox Laboratories Ltd (Crumlin, Co Antrim, UK).
NEFA kits were supplied by Wako chemicals GmbH (Nissanstr. 2, W-4040 NEUSS 1, Germany).
Bovine xanthine oxidase was supplied by Biozyme (Gwent, South Wales, U.K.).
All other chemicals and reagents were supplied by Sigma (St. Louis, Missouri, USA).

2.2. PREPARATIVE TECHNIQUES

Lipoprotein fractions VLDL, HDL and HDL_3 were isolated by standard precipitation techniques. Chylomicrons, VLDL subfractions and LDL subfractions were isolated by cumulative flotation ultracentrifugation.

2.2.1. Preparation of lipoprotein fractions

Serum was used for VLDL isolation because plasma contains high levels of fibrinogen which may interfere with the pelleting of VLDL. Plasma was preferred for HDL isolation because of the preservative properties of EDTA.

2.2.1.1. Very Low Density Lipoprotein

VLDL can be precipitated by SDS to yield results which correlate well with those obtained by ultracentrifugation (Ononogbu and Lewis, 1976).

Reagents

1. 10% SDS w/v in 0.15 M NaCl.
2. 1% SDS w/v in 0.15 M NaCl.

Procedure

75 µl of 10% SDS was added to 1ml serum, mixed and incubated at 37°C for 2 hours. The mixture was centrifuged at 10,000 g for 30mins at 10°C. The pellet was redissolved in 1 ml of 1% SDS at 37°C and stored at -20°C prior to assay.

2.2.1.2. High Density Lipoprotein

Chylomicrons, VLDL and LDL were precipitated and the remaining HDL in the supernatant was isolated. HDL_2 was precipitated in a second step and HDL_3 was isolated in the supernatant. HDL_2 cholesterol and triglyceride were estimated by subtracting HDL_3 levels from total HDL levels (Gidez et al.,1983).

Reagents

1. Manganese Chloride, 1.06 M.
2 Heparin (from porcine intestinal mucosa)(Sigma).
3 Add 1 and 2 to form heparin -$MnCl_2$ (25,000 units heparin /ml of $MnCl_2$).
4 Dextran Sulphate: 1.43% w/v in 0.15 M NaCl.

Procedure

1 ml of 0.15 M NaCl and 0.2 ml of Heparin-$MnCl_2$ were added to 1 ml plasma, mixed and incubated at room temperature for 10 mins. The mixture was centrifuged at 1000g at 4°C for 1 hour. 1.0 ml of supernatant (HDL) was added to 0.1 ml dextran sulphate, mixed and incubated for 20 mins at room temperature and spun as before for 30 mins. The supernatant (HDL_3) was removed and stored at -20°C prior to assay.

2.2.1.3. Low Density Lipoprotein

LDL triglyceride and cholesterol were calculated by subtraction of the sum of HDL and VLDL cholesterol and triglyceride from total plasma cholesterol and triglyceride respectively.

2.2.2. Cumulative flotation ultracentrifugation: preparation of chylomicrons, VLDL subfractions and LDL subfractions

Principle

This method is a modification of the density gradient ultracentrifugation procedure used to isolate LDL subfractions described by Lindgren et al. (1972). Modifications to this LDL subfractionation procedure have been made to permit isolation of chylomicrons, three VLDL subfractions and three LDL subfractions. The flotation rates of these lipoproteins are shown in Table 2.1.

Table 2.1. Flotation rates for chylomicrons, VLDL and LDL subfractions.

LIPOPROTEIN SUBFRACTION	SVEDBERG FLOTATION RATE (S_f)
Chylomicrons	>400
VLDL-1	100-400
VLDL-2	60-100
VLDL-3	20-60
LDL-1	12-20
LDL-2	6-12
LDL-3	0-6

For each lipoprotein subfraction, the angular velocity (w^2t) is calculated for transit of each lipoprotein from maximum radius to minimum radius for each density solution as it floats to the top of the tube, and added to give a total angular velocity (Σw^2t), from which the run speed and run time is calculated.

Total angular velocity is calculated as follows:

$$\sum_{j=1}^{h} w^2 t_j = \sum_{j=1}^{h} (\ln r_{j+1} - \ln r_j) \, / \, \frac{h_s (S_j - \sigma) S_f}{h_j (S_s - \sigma)}$$

where,

S_f	Svedberg flotation rate of the lipoprotein
S_s	Standard density (= 1.063)
S_j	density of solution in intervals
h_s	standard viscosity (= 1.158)
h_j	viscosity of each solution in intervals
σ	hydrated density of lipoprotein
r_{j+1}	maximum radius from the centre of the rotor to the solution intervals
r_j	minimum radius from the centre of the rotor to the solution intervals

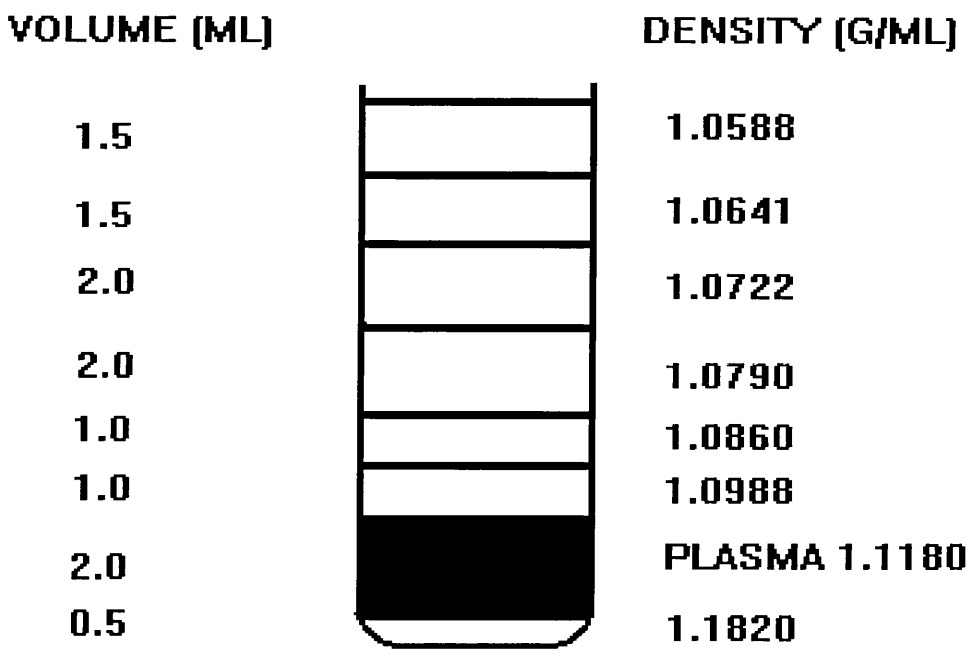
From the formula below, the rotor speed (rpm) and spin time can be calculated.

$$\text{Angular velocity, } w^2t = (\text{radian/seconds})^2 \times \text{seconds}$$
$$= (\text{rpm}/60 \times 2\pi)^2 \times \text{seconds}$$

Method

Blood was collected in vacutainers containing EDTA, and plasma was separated by centrifugation at 1800 rpm for 20 mins at room temperature. Plasma was adjusted to 1.118 g/ml by adding NaCl (0.171g per ml of plasma). Density gradients were prepared from two stock solutions: 1.006g/ml NaCl and 1.182 g/ml NaCl/NaBr and checked using a density meter. 2ml of the density adjusted plasma was layered on 0.5ml of 1.182g/ml solution. On top of the plasma was layered 1ml of 1.0988g/ml, followed by 1ml of 1.086g/ml, 2ml of 1.079g/ml, 2ml of 1.0722g/ml, 1.5ml of 1.0641g/ml and finally 1.5 ml of 1.0588g/ml density solution. The layering of the density solutions is depicted in Figure 2.1.

Fig. 2.1. Formation of the density gradient in the ultracentrifuge tubes.



Centrifuge tubes were coated with polyvinyl alcohol prior to use to enable the density solutions to gravity feed down the tubes (Holmquist, 1982). The tubes were centrifuged in a SW41 rotor in an L8-70 ultracentrifuge at 23^{o}C.

The angular velocity, rotor speed, and run times are tabulated below in Table 2.2. Times are given from switch on to switch off drive power and corrected for previous runs and acceleration and deceleration times. Maximum acceleration was used but the brake was not used. For lower expected values of triglyceride-rich lipoproteins 0.5ml of chylomicrons, VLDL-1, -2 and -3 were taken. Where higher values of triglyceride-rich lipoproteins were expected (in view of postprandial concentrations), run times for chylomicrons, VLDL-1, -2 and -3 were adjusted slightly to allow a 1.0ml (more dilute) sample volume and subsequently the run time for LDL-1 would also differ. Each fraction , 0.5ml or 1.0ml for chylomicrons VLDL-1, -2 and -3, and 1ml for LDL-1, -2 and -3 was carefully aspirated from the top of the tube and density 1.0588g/ml salt solution was used to refill the tube before the next run.

Table 2.2. The angular velocity, rotor speed, and run times required to isolate chylomicrons, VLDL subfractions and LDL subfractions.

Lipoprotein	Angular velocity ($\Sigma w^2 t$) $\times 10^{10}$	Rotor speed (rpm)	Run time (Hrs:mins)	Volume collected (ml)
Chylomicrons	1.46 (1.35)	15K (15K)	1:33 (1:26)	0.5 (1.0)
VLDL-1	4.34 (4.01)	28K (28K)	1:19 (1.13)	0.5 (1.0)
VLDL-2	3.82 (3.52)	28K (28K)	1:09 (1.03)	0.5 (1.0)
VLDL-3	17.4 (15.9)	17K (17K)	15:16 (13:56)	0.5 (1.0)
LDL-1	13.1 (15.4)	39K (39K)	2:06 (2:28)	1.0
LDL-2	29.8	37K	5:26	1.0
LDL-3	41.7	28K	13:28	1.0

Values are for 0.5ml collection volumes for chylomicrons, VLDL-1, -2 and -3 and 1.0ml collection volumes for LDL-1, -2 and -3. In brackets are values for when 1.0ml collection volumes for chylomicrons, VLDL-1, -2 and -3 were taken.

2.3. ANALYSIS OF SERUM AND LIPOPROTEIN FRACTIONS

Cholesterol, triglyceride, phospholipid, free cholesterol, glucose and NEFA were measured by colourimetric assay using commercial kits on an Abbott VP supersystem autoanalyser. Apo AI and Apo B in whole serum were measured by immunoturbidometric assay using commercial kits on the Abbott VP supersystem autoanalyser. Insulin was assayed by radioimmunoassay. Lipoprotein protein was measured using a Lowry procedure or a more sensitive modified Lowry procedure. Apo B was measured in VLDL and LDL subfractions by precipitation of Apo B by addition of isopropanol, followed by protein measurement in the supernatant and the apo B protein concentration was calculated by subtracting the supernatant protein content from the total protein content. Data for apo B (in serum), apo AI, glucose, insulin, and NEFA were provided from other laboratory researchers using the kits specified in the 'reagents' section, and therefore detailed methodology has been omitted. The inter-assay coefficient of variability for these kits was 5%, except for insulin which was 10%.

Abbott vp supersystem autoanalyser

(Abbott Diagnostic Division, Maidenhead, UK)

50µl of blank (salt solution), sample, standards and controls were placed into sample cups. Samples were mixed with reagent in the multi-cuvette according to a pre-

programmed dilution ratio. For cholesterol, triglyceride, free cholesterol and phospholipid assays, the samples, standards and controls were incubated for 12 mins at 37°C and absorbances were read at 500nm. Quality controls were sera of known lipid concentration.

2.3.1. Triglyceride

The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator was a quinoneimine formed by 4-aminophenazone and 4-chlorophenol under catalytic influence of H_20_2. The standard used was glycerol (50mg/ml). The inter-assay coefficient of variability was 5%.

Triglyceride + $3H_2O$ $\rightarrow$ (lipases) $\rightarrow$ glycerol + 3RCOOH

Glycerol + ATP $\rightarrow$ (glycerol kinase) $\rightarrow$ glycerol-3-phosphate (G-3-P)+ ADP

G-3-P +O_2 $\rightarrow$ (glycerol-3-phosphate oxidase) $\rightarrow$ dihydroxyacetone phosphate + H_2O_2

2 H_2O_2 + 4-aminophenazone + 4 chlorophenol $\rightarrow$ (peroxidase) $\rightarrow$ $2H_2O$ + HCL + 4-(p-benzoquinone-mono-imino)-phenazone.

2.3.2. Cholesterol

Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The standard used was cholesterol (50mg/dl). The inter-assay coefficient of variability was 5%.

Cholesterol ester $\rightarrow$ (cholesterol esterase) $\rightarrow$ Cholesterol + RCOOH

Cholesterol + O_2 $\rightarrow$ (cholesterol oxidase) $\rightarrow$ Cholestene-3-one + H_2O_2

Phenol + $2H_2O_2$ + 4-aminophenazone $\rightarrow$ (peroxidase)$\rightarrow$ 4-(p-benzoquinone-mono imino) phenazone + 4 H_2O.

2.3.3. Free cholesterol

Free cholesterol was determined after enzymatic oxidation. The indicator was 4-(P-benzoquinone-mono-imino) Phenazone, formed from H_2O_2 and 4-aminophenozone in the presence of phenol and peroxidase. The standard used was cholesterol (50mg/dl). The inter-assay coefficient of variability was 5%.

Cholesterol $+O_2 \rightarrow$ (cholesterol oxidase) $\rightarrow$ 4,-cholestenone $+H_2O_2$

$2H_2O_2$ + 4-amino phenazone + phenol $\rightarrow$ (peroxidase) $\rightarrow$ 4-(p-benzoquinone-mono-imino) phenozone $+4H_2O$.

2.3.4. Cholesterol ester

Cholesterol ester (mmol\l) was calculated as (total cholesterol - free cholesterol). Cholesterol ester (mg/dl) was calculated as (total cholesterol-free cholesterol) × 1.68 to account for the mass of the ester.

2.3.5. Phosholipid

Phospholipid was determined by enzymatic hydrolysis and oxidation . The indicator was 4- (P-benzoquinone-mono-imino) phenazone formed from H_20_2 and 4- amino phenazone in the presence of phenol and peroxidase. The standard used was choline chloride (75 mg/dl). The inter-assay coefficient of variability was 5%.

Phospholipid + $H_2O \rightarrow$ (phospholipase D) $\rightarrow$ choline + phosphatidic acids.

Choline $+2O_2 + H_2O \rightarrow$ (choline oxidase) $\rightarrow$ betaine + $2H_2O_2$

H_2O_2 + 4-aminophenazone + phenol $\rightarrow$ (peroxidase) $\rightarrow$ 4(p-benzoquinone-mono-imino) phenazone + $4H_2O$.

2.3.6. Protein determination

2.3.6.1. Lowry method

Lowry method was used for lipoprotein subfractions, which were diluted if necessary prior to assay (Lowry et al., 1951). The inter-assay coefficient of variability was <10%.

Working Reagents

A. 2% w/v Na_2CO_3 in 0.1M NaOH containing 0.02 % NaK tartrate w/v and 0.01 % w/v $CuSO_4$. 5% SDS w/v was added to prevent turbidity.

B. Folin reagent (1:1 with water).

C. Working standard curve : 0-0.4 mg/ml BSA made up in salt solution .

Method

100 µl of lipoprotein sample was added to 500 µl reagent A and incubated for 10 minutes at room temperature. 50 µl of reagent B was added, mixed and incubated for 1 hour at 37°C. Absorbances were read at 690 nm.

Sensitive Lowry method

This is a more sensitive Lowry procedure often used for lipoprotein subfractions such as fasting chylomicrons, VLDL-1 and VLDL-2. It was also used for measuring apo B concentrations in lipoprotein subfractions (Markwell et al., 1979). The inter-assay coefficient of variability was <10%.

Working Reagents

A. 2% $NaCO_3$ w/v, 0.4% NaOH w/v, 0.16 % Natartrate w/v, 5% SDS w/v, 0.04% $CuSO_4.5H_20$ w/v.

B. Folin (1:1 with water).

C. Working standard curve: 0-0.1 mg/ml BSA made up in salt solution.

Method

100 µl of lipoprotein sample was added to 300 µl of reagent A, mixed and incubated for ten minutes at room temperature. 30 µl of reagent B was added and incubated for 1 hour 37°C. Absorbances were read at 690nm.

2.3.7. Isopropanol precipitation for determination of apolipoprotein B in lipoprotein subfractions

Apo B was separated from the other apolipoproteins in the lipoprotein subfractions by precipitation in isopropanol and its concentration was measured as the difference between total protein and the proteins assayed in the supernatant (soluble protein) (Egusa et al.,1983). The total protein and supernatant protein were measured either by a standard Lowry procedure or by the modified senstive Lowry procedure. Apo B was measured in VLDL-1, -2, -3 and LDL-1 from fasting plasma. Concentrations of apo B in chylomicrons and non-apo B protein in LDL-2 and -3 were very small and therefore concentrations of apo B were not determined in these fractions. The inter-assay coefficient of variability was <10%.

Method

200µl of lipoprotein sample was added to 200µl of isopropanol, mixed vigorously for one minute and incubated overnight at 4°C. The supernatant was removed for protein determination using the Lowry or Markwell procedure.

2.4. INSULIN RESISTANCE AND β-CELL FUNCTION

Insulin resistance and β-cell function were calculated from fasting glucose and insulin concentrations using the computer-solved homeostasis model assessment (HOMA) method as described by Matthews et al. (1985). The estimate of insulin resistance has been shown to correlate with those obtained using the euglycaemic and hyperglycaemic clamp and the estimate of β-cell function has been shown to correlate with those obtained using the hyperglycaemic clamp.

$$\text{Insulin resistance} = \text{fasting insulin} / 22.5\ e^{-\ln \text{fasting glucose}}$$

$$\beta\text{-cell function (\%)} = 20 \times \text{insulin} / (\text{glucose} - 3.5)$$

2.5. MEASUREMENT OF HUMAN ANTI-(BOVINE XANTHINE OXIDASE) IgM AND IgG CLASS ANTIBODIES

2.5.1. Preparation of pure bovine xanthine oxidase

Bovine xanthine oxidase was either supplied commercially (Biozyme), or freshly prepared by J.Khan (Bath University) according to the method of Nakamura and Yamazaki (1982). Xanthine oxidase was dialysed in 50mM sodium carbonate to remove aluminium sulphate present in the crude enzyme, and protein concentration was estimated (Lowry method).

2.5.2. Enzyme Linked Immuno-sorbent Assay (ELISA)

Principle

1. Sensitisation of the plate. Antigen coating buffer is incubated at 4°C over night or for 4 hours at 37°C Some of the antigen is absorbed onto the plastic surface of the plate and the rest remains free.
2. Washing. PBS\Tween buffer is used in order to wash away any free antigen which is not bound to the plate.
3. Addition of the test antibody. The test antibody once added will bind to the antigen.
4. Washing. Unbound proteins are washed away.
5. Addition of a ligand. A ligand is added which is a molecule that can bind to the test antibody and is covalently coupled to an enzyme such as peroxidase.
6. Washing. Free ligands is washed away.
7. Addition of chromogen. The bound ligand is visualised by the addition of a chromagen, a colourless substrate which is acted upon by the enzyme portion of the ligand to produce a coloured end product.
8. Stopping the reaction. The reaction is stopped by the addition of 1M H_2SO_4 and the amount of test antibody is measured by assessing the amount of coloured end-product by scanning the optical density of the plate.

Method

Xanthine oxidase (0.02mg/ml) in 50mM sodium carbonate, pH 9.6, was added to each well of a 96-well microtitre plate (Flow Labs. Irvine, Scotland) and incubated overnight at 4°C. The wells were washed by incubation at room temperature (60 minutes) with 3 successive portions (250µl/well) of phosphate buffered saline containing 0.1% Tween 20 and then incubated with serum samples (100µl, diluted 400-fold in the same buffer) for 90 minutes at 37°C. The plates were washed as above and 100µl of goat anti-(human IgM or IgG) IgG Horse Radish Peroxidase conjugate (diluted 1000 fold in phosphate buffered saline containing 0.1% Tween 20) was added to each well.

The wells were incubated for 2 hours at room temperature, washed three times as above and incubated with staining buffer (100µl/well) for 20-30 minutes until a blue colour developed. The reaction was stopped by the addition of 50µl of 1M sulphuric acid, and absorbance was recorded at 450nm. The staining buffer contained 1% 3,3',5,5' tetramethylbenzidine in dimethylsulphoxide, diluted 100 fold in 0.1M sodium acetate /citric acid pH 6.0 and 30% hydogen peroxide (0.1% µl/ml) which was added immediately before use.

Titres are quoted as percentage absorbance shown by standard high titre pooled human serum, assayed on the same plate. These have been converted to concentration of antibody (µg/ml) in Chapter 6. All values are means of triplicate determinations and in comparative studies, patient and control samples were assayed on the same plate. The inter-assay coefficient of variability was <10%.

2.6. STATISTICS

Results in this thesis are expressed as mean and standard error of the mean (SEM). Levels of significance for statistical analyses was $p \leq 0.05$.

Anti-(XO) antibody levels were compared in populations using the Kolmogorov-Smirnov two-tailed non-parametric test. The test is sensitive to any kind of difference in the distributions from which the two samples were drawn-differences in location (central tendency), in dispersion and in skewness. This procedure calculates the maximum distance between the cumulative distribution functions (Dmax) of the two samples. If this distance is large enough, the hypothesis that the distributions are the same is rejected.

For lipid, lipoprotein and metabolite measurements, normally distributed data and

data normally distributed after log-transformation were analysed using Student's t-tests for unpaired or paired data. Non-parametric data were analysed using the Mann-Whitney test for unpaired data or the Wilcoxon test for paired data.

Correlations were made using Pearson's correlation test for normally distributed and data normally distributed after log-transformation, and the Spearman rank correlation test for non-parametric data. Multiple linear regression analysis was also used in Chapter 3.

Oxstat V (Holman, Oxford) was the statistical software used for all analyses.

CHAPTER 3

FASTING AND POSTPRANDIAL LIPAEMIA IN NIDDM

3.1. INTRODUCTION

It can be postulated that a range of quantitative and qualitative changes in fasting and postprandial lipoprotein subfraction composition, not shown in basic lipoprotein levels, may be present in diabetes and contribute to excess risk of CHD. Until recently, the majority of investigations have focused on fasting dyslipidaemia in NIDDM. However, since the majority of life is spent in the postprandial state, investigations into postprandial lipaemia would seem worthwhile. Furthermore, if fasting triglycerides are raised in patients with NIDDM, then more of life may be in the postprandial state in these patients. In past studies, triglyceride has been excluded as a risk factor for CHD because in multivariate analysis triglyceride is eliminated by its relationship to multiple other risk factors including HDL cholesterol. However it has recently been shown that maximum triglyceride increase and magnitude of postprandial lipaemia was a better predictor of CHD than HDL cholesterol (Patsch et al.,1992).

The aim of this chapter is to investigate postprandial lipaemia in hyperlipidaemic patients with NIDDM compared to age- and gender-matched normolipidaemic non-diabetic controls.

3.2. METHODS

3.2.1. Study Population

a) Ten patients with moderate dyslipidaemia but satisfactory glycaemic control (Table 3.1 and 3.2), and treated with diet and glibenclamide (5-15 mg) daily were studied.

b) Ten age- and gender-matched non-diabetic normolipidaemic control subjects were obtained from the same geographical area (Tables 3.1 and 3.2).

Ethical approval was obtained from the institutional review committee (Bath District Research Ethics Committee).

Table 3.1. Inclusion and exclusion criteria for diabetic patients and controls.

	INCLUSION	EXCLUSION
NIDDM	Fructosamine < 340 μmol/L Cholesterol: 5.2 -8.5 mM Triglyceride: 1.8-4.5 mM	Metformin, Cyclical HRT, Renal and Hepatic disease, Other significant disease
CONTROL	Triglyceride: < 2.0 mM	

Table 3.2. Demographic data for patients and controls.

	NIDDM	CONTROL
NUMBER	10	10
AGE (yrs)± SEM	53.6 ± 2.91	53.2 ± 3.42
GENDER	8 male, 2 female	8 male, 2 female
BMI ($kg.m^{-2}$) ± SEM	27.8 ± 0.89	24.9 ± 0.79 a

a: $p<0.05$

3.2.2. Protocol

1. Fulfill entry criteria.
2. Written informed consent.
3. 8 weeks run-in on placebo (single-blind) with stable glycaemic control and lipid parameters.
4. 0800 hours: Fasting blood sample
5. 0815 hours: Standard low fat breakfast followed by normal daily activity.
6. 1200 hours: Standard low fat lunch provided.
7. 1800 hours: Admitted for overnight study.
8. 1845 and 1855 hours: Blood samples taken to check that lipid levels were similar to fasting levels.
9. 1900 hours: Test meal: mixed meal, high fat (50g fat, 1000 calories).
10. Blood samples were taken at hourly intervals for 12 hours after the test meal.

3.2.3. Measurements

Cholesterol and triglyceride in serum and serum lipoproteins (VLDL, HDL, HDL_2 and HDL_3), serum apolipoproteins (AI and B), glucose, insulin and NEFA were measured at every time interval (data supplied by C.Stirling and co-workers). Lipoprotein

subfractions were isolated from plasma sampled at fasting, and at 3 and 8 hours after the test meal. Measurements for each subfraction included total cholesterol, triglyceride, phospholipid, free cholesterol, esterified cholesterol and total protein. The mass of each fraction was calculated as the sum of the latter five components. Composition of these fractions was expressed as a percentage of total mass. Fasting apo B concentrations were determined in VLDL-1, -2 and -3 and LDL-1 fractions only (see Methods 2.3.7). Cholesterol and triglyceride values for VLDL and LDL were calculated as the sum of these lipid components in VLDL and LDL subfractions respectively. Areas under curves (AUCs) were calculated using the trapezoid rule. Incremental areas under curves (IAUCs) were calculated by subtracting the baseline value, extrapolated over time, from the AUC value. Insulin resistance and β-cell function were assessed using the HOMA method (see Methods section).

3.2.4. Statistics

Comparisons between groups (diabetic patients vs controls) were made using an unpaired t-test. Comparisons within subject groups (fasting vs postprandial) were made using a paired t-test. Measurements for triglyceride, glucose, insulin, β-cell function % and insulin resistance were log-transformed prior to analysis. Correlations were made using Pearson's correlation test and multiple linear regression analysis. Non-parametric data which could not be log-transformed (IAUC for triglyceride, glucose, and insulin) were compared by Mann-U-Whitney test (unpaired), Wilcoxon test (paired). Data are presented as mean ± SEM.

3.3. RESULTS

3.3.1. Fasting lipids, lipoproteins and metabolites in diabetic patients and controls

Fasting lipids, lipoproteins, insulin, NEFA and glucose results are shown in Table 3.3. Serum, LDL and VLDL triglyceride, VLDL cholesterol and apo B were significantly elevated in diabetic patients compared to controls. Since serum and LDL cholesterol were similar in both subject groups, much of the difference in apo B levels is likely to be accounted for by the raised levels of VLDL in diabetic patients. However, elevated apo B

levels could also be accounted for in part by an increase in small, dense LDL. Additionally an increase in VLDL triglyceride and cholesterol may occur without an increase in VLDL apo B when bigger VLDL molecules are synthesised. HDL and HDL_2 cholesterol were significantly lower in diabetic patients compared to controls which were reflected by significantly reduced levels of apo AI. HDL_3 cholesterol was also reduced in diabetic patients, although not significantly so. In addition, diabetic patients had raised levels of HDL triglyceride which were reflected in an increase in HDL_3 triglyceride ($p=0.07$).

Diabetic patients had significantly higher levels of glucose and NEFA compared to controls. Levels of insulin were also raised in diabetic patients, but the results did not reach statistical significance.

Table 3.3. Lipids, lipoproteins, apolipoproteins and other clinical data in diabetic patients and controls.

	NIDDM	Controls	
Cholesterol (mM)	6.56±0.32	6.00±0.44	
Triglyceride (mM)	3.48±0.27	1.44±0.14	c
VLDL cholesterol (mM)	1.17±0.20	0.46±0.08	b
VLDL triglyceride (mM)	2.08±0.23	0.61±0.13	c
LDL cholesterol (mM)	3.25±0.34	2.79±0.29	
LDL triglyceride (mM)	0.33±0.03	0.23±0.03	a
HDL cholesterol (mM)	0.94±0.05	1.75±0.18	c
HDL triglyceride (mM)	0.25±0.02	0.19±0.02	b
HDL_2 cholesterol (mM)	0.34±0.03	0.82±0.16	b
HDL_2 triglyceride (mM)	0.07±0.01	0.06±0.02	
HDL_3 cholesterol (mM)	0.61±0.05	0.93±0.10	b
HDL_3 triglyceride (mM)	0.21±0.03	0.13±0.02	
Apo B (mg/dl)	162±8.0	75.0±7.2	c
Apo AI (mg/dl)	150±4.1	182±8.0	b
Glucose (mM)	10.1±0.67	5.49±0.10	c
Total insulin (mU/l)	11.9±3.35	9.21±1.44	
NEFA (mM)	0.81±0.06	0.57±0.07	a

All values are mean ± SEM.
a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

3.3.2. Fasting chylomicrons, VLDL subfractions and LDL subfractions in diabetic patients and controls

Concentrations of mass for chylomicrons, VLDL-1, -2 and -3, LDL-1, -2 and -3 are shown in Fig. 3.1, and lipid and protein components of these lipoproteins are shown in Table 3.4. Chylomicrons, VLDL-1, -2 and -3 were significantly elevated in diabetic patients compared to controls as were the triglyceride components of chylomicrons and all the lipid and protein components of the VLDL subfractions. Raised levels of apo B in VLDL subfractions indicate that the number of particles of these subfractions was higher in diabetic patients than in controls. Diabetic patients had a more polydisperse LDL subfraction profile than control subjects. LDL-3 mass and all its lipid and protein components were significantly higher in diabetic patients compared to controls. There were no significant differences in LDL-1 and -2 mass, although LDL-1 mass tended to be higher and LDL-2 mass tended to be lower in diabetic patients. LDL-2 was free cholesterol significantly higher in controls.

Results from analyses of lipid and protein composition of chylomicrons and VLDL subfractions and LDL subfractions (expressed as a percentage of total mass) are shown in Table 3.5. Percentage esterified cholesterol in VLDL-1 and free cholesterol in VLDL-2, -3, LDL-1, -2 and -3 were significantly reduced in diabetic patients compared to controls. This was reflected by an increase in % triglyceride in VLDL-1, -2 and -3, LDL-1 and -2, although only reaching statistical significance for LDL-2. Diabetic patients also had significantly higher % triglyceride in chylomicrons compared to controls, indicative of larger particle size.

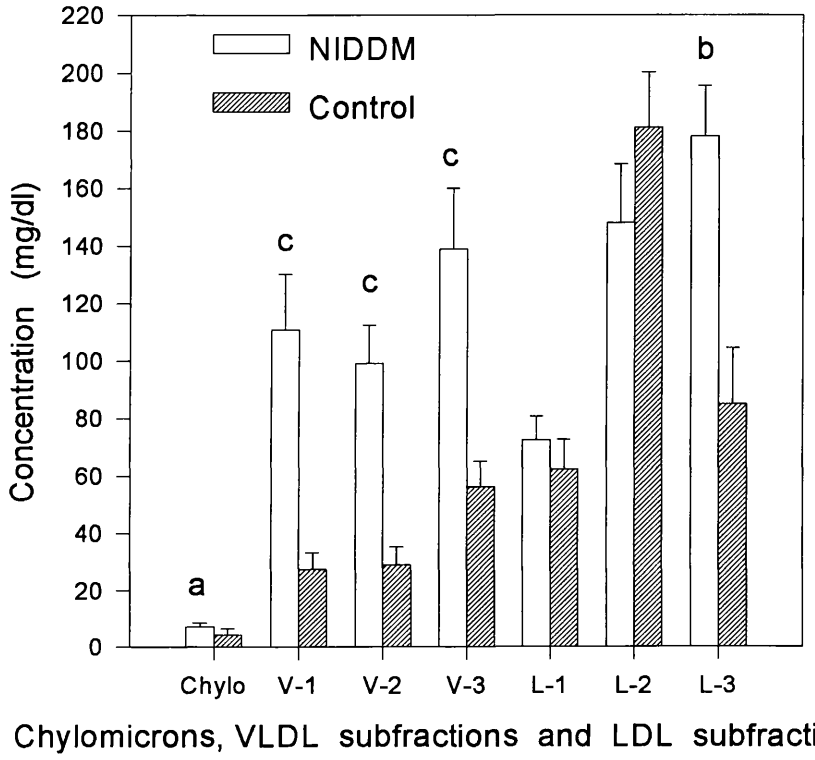


Fig. 3.1. Concentration of chylomicrons (chylo). VLDL subfractions (V-1, V-2 and V-3) and LDL subfractions (L-1, L-2 and L-3) in NIDDM patients and controls.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Table 3.4. Fasting concentrations of lipid and protein components of chylomicrons, VLDL subfractions and LDL subfractions in diabetic patients and controls.

	Chylomicrons		VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr
CH	0.64± 0.20	0.50± 0.32	8.35± 1.61	2.93± 0.93 b	11.3± 2.08	3.51± 0.66 b	25.8± 4.94	11.3± 1.93 a	21.5± 2.88	19.0± 3.56	48.2± 7.42	61.6± 6.54	56.2± 5.67	27.2± 6.19 b
TG	5.19± 1.06	2.31± 1.34 a	73.7± 12.7	17.4± 3.85 c	54.8± 6.08	15.9± 4.39 c	55.1± 6.90	20.7± 4.15 c	11.9± 1.19	9.16± 1.51	10.2± 1.43	7.98± 0.88	7.25± 0.79	3.49± 0.74 b
PL	0.61± 0.13	0.71± 0.31	14.6± 3.20	3.65± 0.93 b	15.8± 2.82	4.69± 1.00 b	24.2± 4.36	11.4± 1.76 a	14.6± 1.54	13.7± 2.71	31.0± 5.00	39.4± 3.78	32.9± 3.75	17.2± 4.05 a
FC	0.19± 0.06	0.24± 0.16	4.90± 0.92	1.70± 0.52 b	5.10± 0.57	1.97± 0.36 c	10.5± 1.64	5.23± 0.80 a	7.13± 0.78	7.04± 1.41	13.5± 1.87	19.1± 1.82 a	13.2± 1.34	7.98± 1.82 a
EC	0.75± 0.26	0.44± 0.26	5.80± 1.26	2.07± 0.70 a	10.4± 2.93	2.58± 0.52 a	25.7± 5.71	10.2± 1.93 a	24.2± 3.60	20.0± 3.68	58.4± 9.44	71.3± 8.29	72.1± 7.38	32.4± 7.41 b
Prot	0.37± 0.09	0.63± 0.32	11.7± 1.80	2.89± 0.93 c	13.1± 1.82	3.90± 0.83 c	23.3± 2.74	8.65± 1.26 b	14.7± 1.70	12.2± 1.59	35.4± 3.14	42.9± 5.57	52.4± 4.91	23.8± 6.08 b
Apo B			5.00± 0.94	1.82± 0.78 a	5.37± 0.94	2.22± 0.54 b	14.9± 2.84	6.39± 1.05 a	13.2± 1.65	10.7± 1.45				
Mass	7.12± 1.39	4.33± 2.30 a	110± 19.3	27.5± 5.64 c	99.2± 13.3	29.0± 6.14 c	139± 21.1	56.2± 8.89 c	72.5± 8.23	62.1± 10.6	148± 20.3	181± 19.1	178± 17.6	84.8± 19.7 b

All concentrations are mean ± SEM (mg/dl).

NIDD: NIDDM; Contr: Controls; CH: cholesterol; TG: triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Table 3.5. Fasting composition of chylomicrons, VLDL subfractions and LDL subfractions in diabetic patients and controls.

	Chylomicrons		VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr
CH	9.04± 1.56	11.4± 2.31	7.37± 0.44	10.7± 1.45 a	10.9± 0.54	13.9± 1.83	18.2± 0.71	20.5± 1.30	29.1± 0.93	29.8± 0.81	31.8± 0.72	34.1± 0.76 a	31.5± 0.40	31.1± 1.40
TG	71.6± 3.08	44.6± 6.83 a	67.2± 1.41	59.1± 5.22	56.9± 1.58	48.6± 6.08	40.3± 1.37	34.8± 3.42	16.8± 0.90	15.0± 0.84	7.03± 0.45	4.50± 0.40 c	4.06± 0.23	6.57± 2.96
PL	9.84± 2.83	18.1± 7.22	12.0± 1.26	16.7± 2.98	15.0± 1.27	18.1± 2.77	17.3± 1.08	20.9± 1.19 a	20.6± 1.04	21.9± 0.80	20.3± 0.72	22.0± 0.54	18.4± 0.57	20.0± 0.64
FC	3.34± 0.75	4.82± 2.00	4.35± 0.19	5.78± 0.91	5.33± 0.25	7.64± 0.96 a	7.60± 1.34	9.75± 0.55 b	9.91± 0.19	11.1± 0.48 a	9.07± 0.31	10.7± 0.41 b	7.46± 0.27	9.15± 0.62 a
EC	9.56± 1.94	11.0± 1.56	5.08± 0.68	8.30± 1.36 a	9.29± 1.24	10.4± 1.61	17.9± 1.08	18.0± 1.52	32.3± 1.78	31.3± 1.37	38.2± 1.21	39.3± 1.48	40.4± 0.58	36.9± 1.72
Prot	5.63± 1.02	21.5± 4.96 b	11.3± 0.99	10.1± 1.22	13.4± 0.87	15.2± 2.22	16.9± 1.30	16.5± 1.17	20.5± 0.81	20.7± 0.80	25.5± 1.61	23.5± 1.08	29.7± 0.91	27.4± 0.99

All values are mean % of total mass ± SEM.

NIDD: NIDDM; Contr: Controls; CH: cholesterol; TG: triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

A measurement of qualitative changes in lipoproteins, lipoprotein subfractions and metabolites is shown in Table 3.6. The ratio of LDL/HDL cholesterol was significantly increased in diabetic patients. HDL_2/HDL_3 ratio was substantially reduced (by 39%) in diabetic patients, although the results did not reach statistical significance. Furthermore, HDL, HDL_2 and HDL_3 were triglyceride-enriched in diabetic patients compared to controls as shown by their significantly higher triglyceride/cholesterol ratio.

Percent VLDL-3 in total VLDL (%VLDL-3/ΣVLDL) was significantly reduced in diabetic patients compared to controls, mirrored by a substantial increase in %VLDL-1/ΣVLDL (38%). These findings suggest that diabetic patients had a smaller proportion of small VLDL and consequently a greater proportion of large VLDL. Percent LDL-3 in total LDL (%LDL-3/ΣLDL), was significantly elevated in diabetic patients, suggesting that diabetics had a greater proportion of small, dense LDL compared to controls.

The ratio of non-apo B protein to apo B protein was significantly elevated in VLDL subfractions in diabetic patients compared to controls which was reflected by an increase in triglyceride/apo B ratio in these subfractions in diabetic patients, albeit only significantly so for VLDL-2. These ratios are indicative of lipoprotein particle size and therefore the differences suggest that diabetic patients have larger particle size within each of the VLDL subfractions than controls.

β-cell function was reduced and insulin resistance was elevated in diabetic patients compared to controls, although the latter difference was not significance.

Table 3.6. Qualitative lipoprotein, lipoprotein subfraction and metabolite parameters in diabetic patients and controls.

	NIDDM	Controls	
%VLDL-1/ ΣVLDL	31.0±3.37	22.5±2.58	
%VLDL-2/ ΣVLDL	28.0±0.81	24.8±1.96	
%VLDL-3/ ΣVLDL	41.0±3.54	52.7±2.83	a
%LDL-3/ΣLDL	45.1±2.20	23.7±3.60	c
LDL chol/ HDL chol	3.52±0.37	1.89±0.34	b
HDL_2/ HDL_3 chol	0.60±0.09	0.99±0.26	
HDL trig/chol	0.28±0.03	0.12±0.02	b
HDL_2 trig/chol	0.21±0.03	0.10±0.03	b
HDL_3 trig/chol	0.38±0.08	0.15±0.02	a
Insulin resistance	5.23±1.40	2.23±0.34	
β-cell function %	40.2±12.3	96.5±16.7	b
VLDL-1 trig/apo B	19.4±4.63	13.4±2.92	
VLDL-2 trig/apo B	11.9±1.45	8.01±2.03	a
VLDL-3 trig/apo B	4.17±0.45	3.26±0.61	
VLDL-1 non-apoB/apo B	1.74±0.37	0.82±0.14	a
VLDL-2 non-apoB/apo B	1.63±0.32	0.87±0.10	b
VLDL-3 non-apoB/apo B	0.63±0.07	0.39±0.05	a

All values are mean ± SEM.
ΣVLDL: sum of VLDL subfraction mass; ΣLDL: sum of LDL subfraction mass;
chol: cholesterol; trig: triglyceride; non-apo B: non-apo B protein.
a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

3.3.3. Postprandial lipid, lipoproteins and metabolites in diabetic patients and controls

Postprandial responses for lipids, lipoproteins and metabolites are shown in Fig. 3.2-3.5, and AUC and IAUC for these data are shown underneath in Tables 3.7, and 3.9-3.11 respectively. Paired comparisons between fasting levels of these parameters and levels at three hours and eight hours after the test meal within each subject group are shown in Table 3.8.

After the test meal, serum and VLDL triglyceride, VLDL cholesterol and apo B increased in both subject groups respectively (Figs. 3.2 and 3.3). AUC for these parameters and IAUC for VLDL triglyceride were significantly increased in diabetic patients compared to controls (Tables 3.7 and 3.9). Peak triglyceride was delayed in diabetic patients at 5-7 hours compared to 3-5 hours in controls. Peak serum apoB and VLDL cholesterol and

triglyceride levels were very delayed in diabetic patients at approximately 8 hours compared to 4 hours for controls. Comparisons within each subject group (Table 3.8) show that levels of VLDL triglyceride and apo B were significantly higher at 8 hours compared to fasting levels in diabetic patients, whereas in controls there were only significant differences at 3 hours, suggesting that the return from peak to baseline levels for VLDL triglyceride and apo B were slower in diabetic patients compared to controls.

Despite diabetic patients having significantly higher AUC for serum cholesterol (Table 3.7), levels decreased after the test meal in both subject groups (Fig. 3.2). This was particularly apparent in the control group, in which cholesterol levels were significantly reduced at 3 and 8 hours compared to fasting levels (Table 3.8). In addition, reductions in LDL cholesterol at 8 hours were larger and more significant in controls compared to diabetic patients (Table 3.8).

For diabetic patients and controls, HDL, HDL_2 and HDL_3 cholesterol and apo AI levels decreased after the test meal (Figs 3.3 and 3.4). AUC for these parameters were significantly reduced in diabetic patients, but there were no differences in IAUC (Tables 3.9 and 3.10). In both subject groups there were significant reductions in HDL and HDL_3 cholesterol after 8 hours, but there were only significant reductions in HDL_2 cholesterol and apo AI after 8 hours in the control group (Table 3.8).

In both subject groups HDL and HDL_3 triglyceride levels, after an initial decrease, increased, peaked and returned to approximately baseline levels (Figs. 3.3 and 3.4). The AUC for HDL and HDL_3 triglyceride was higher in diabetic patients than controls (reaching significance for HDL_3), but there were no differences in IAUC (Tables 3.9 and 3.10). Furthermore, peak levels for HDL and HDL_3 triglyceride were delayed in diabetic patients. HDL_2 triglyceride increased after the test meal in both diabetic patients and controls, and the AUC, IAUC and peak level for HDL_2 triglyceride was similar in both subject groups (Fig. 3.4, Table 3.10). There were no significant differences between fasting levels and levels at 3 and 8 hours for HDL, HDL_2 and HDL_3 triglyceride in either of the subject groups (Table 3.8).

After the test meal, concentrations of insulin and glucose increased, peaked and returned to the baseline within 12 hours in both subject groups. However peak levels for these parameters were delayed in diabetic patients (Fig. 3.5). AUC for insulin and glucose and IAUC for glucose were significantly higher in diabetic patients compared to controls (Table 3.11). Insulin levels were significantly higher after 3 and 8 hours compared to fasting levels in diabetic patients but only at 3 hours in controls (Table 3.8), indicative of a prolonged insulin response in diabetic patients. Levels of NEFA, after the test meal, initially

decreased and then increased, peaked and returned to below baseline level in both subject groups (Fig. 3.5). Diabetic patients had a significantly raised AUC for NEFA (Table 3.11) and a delayed peak compared to controls.

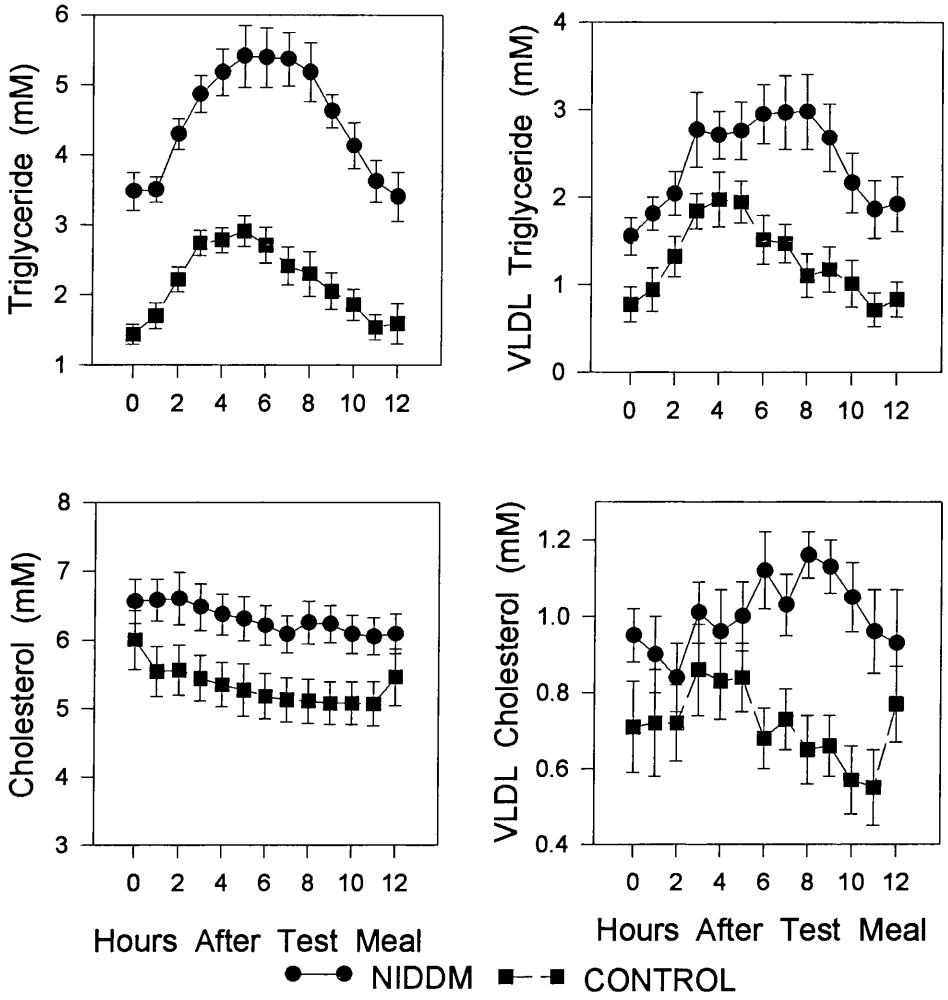


Fig. 3.2 Fasting and postprandial concentrations for serum triglyceride, serum cholesterol, VLDL triglyceride and VLDL cholesterol.

Table 3.7. AUC and IAUC in diabetic patients and controls.

	NIDDM AUC	Control AUC	NIDDM IAUC	Control IAUC
Cholesterol (mM.hrs)	75.5±3.31	63.5±4.02 a	-3.16±6.21	-8.41±1.98
Triglyceride (mM.hrs)	55.0±2.73	26.7±2.18 c	13.2±2.77	9.46±1.15
VLDL triglyceride (mM.hrs)	29.43±2.95	15.8±2.34 c	10.8±1.59	6.54±1.10 a
VLDL cholesterol (mM.hrs)	12.1±0.89	8.56±0.89 a	0.75±0.68	-0.01±0.93

All areas were calculated as a product of concentration (units as in table above) and time (hours) over 12 hours. AUCs are calculated as areas above zero concentration, while IAUCs are calculated as net areas above or below (+ or -) the concentration at basal time in this and subsequent calculations. All values are mean ± SEM.
a: $p<0.05$; c: $p<0.001$

Table 3.8. Comparison of paired data (0hrs vs 3hrs and 0hrs vs 8hrs) within each subject group.

	Diabetic patients			Controls		
	0 hours	3 hours	8 hours	0 hours	3 hours	8 hours
Chol (mM)	6.55± 0.32	6.48± 0.34	6.25± 0.32	6.00± 0.44	5.44± 0.33 b	5.11± 0.32 b
Trig (mM)	3.48± 0.27	4.87± 0.26 c	5.18± 0.42 b	1.44± 0.14	2.74± 0.18 c	2.30± 0.33 b
VLDL trig (mM)	1.55± 0.21	2.77± 0.43 b	2.98± 0.43 c	0.77± 0.20	1.84± 0.20 c	1.10± 0.25
LDL chol (mM)	126± 13.3	116± 12.1	111± 10.6 a	108± 11.2	98.6± 10.5 b	91.8± 9.95 c
Apo B (mg/dl)	126± 7.96	141± 7.50	149± 7.78 a	75.4± 7.24	96.3± 7.25 b	80.8± 7.67
Apo AI (mg/dl)	150± 4.08	145± 4.66	148± 5.15	182± 7.99	179± 6.18	163± 7.10 c
HDL chol (mM)	0.94± 0.05	0.88± 0.05 b	0.76± 0.05 c	1.75± 0.18	1.58± 0.20	1.44± 0.16 c
HDL trig (mM)	0.25± 0.02	0.25± 0.03	0.28± 0.04	0.19± 0.02	0.21± 0.03	0.22± 0.02
HDL_2 chol (mM)	0.34± 0.03	0.34± 0.04	0.29± 0.04	0.82± 0.16	0.70± 0.11	0.67± 0.11 a
HDL_2 trig (mM)	0.07± 0.01	0.08± 0.01	0.08± 0.01	0.06± 0.02	0.08± 0.01	0.09± 0.02
HDL_3 chol (mM)	0.61± 0.05	0.54± 0.03	0.47± 0.04 b	0.93± 0.10	0.88± 0.13	0.77± 0.09 c
HDL_3 trig (mM)	0.21± 0.03	0.18± 0.02	0.21± 0.03	0.13± 0.02	0.13± 0.02	0.13± 0.02
VLDL-1 %trig	67.2± 1.41	66.9± 0.92	65.7± 1.20	59.1± 5.22	64.7± 2.07	62.4± 2.31
VLDL-1 %free chol	4.35± 0.19	4.26± 0.12	3.86± 0.24 a	5.79± 0.91	5.29± 0.61	4.79± 0.59
VLDL-2 %trig	56.9± 1.58	57.1± 1.55	56.6± 1.30	48.6± 6.08	54.7± 3.02	53.9± 1.43
VLDL-2 %free chol	5.33± 0.25	5.79± 0.28	5.32± 0.26	7.64± 0.96	6.22± 1.04	4.95± 0.84 b
VLDL-3 %trig	40.3± 1.37	40.0± 2.41	42.8± 1.41	34.8± 3.42	41.9± 1.65 a	41.8± 1.32
VLDL-3 %free chol	7.60± 0.34	8.06± 0.63	7.31± 0.29	9.75± 0.55	8.39± 0.53 a	8.04± 0.40 b
VLDL-3 %est. chol	17.9± 1.08	15.0± 1.39	14.4± 1.23 a	18.0± 1.52	14.7± 1.26 a	15.3± 0.86
LDL-1 %trig	16.8± 0.90	15.8± 1.03	16.2± 0.96	15.0± 0.84	15.3± 1.63	15.2± 1.33
LDL-1 %free chol	9.91± 0.19	9.27± 0.39	9.58± 0.53	11.2± 0.48	9.51± 0.68 a	9.47± 0.47 b
LDL-2 %trig	7.03± 0.45	7.11± 0.49	6.99± 0.65	4.50± 0.40	4.42± 0.40	5.00± 0.45 b
LDL-2 %free chol	9.07± 0.31	8.76± 0.29	8.17± 0.42	10.7± 0.41	9.65± 0.30 a	9.67± 0.32 b

Table 3.8. (continued)

	Diabetic patients			Controls		
	0 hours	3 hours	8 hours	0 hours	3 hours	8 hours
LDL-3 %trig	4.06± 0.23	4.46± 0.26 a	4.62± 0.30 b	6.57± 2.96	7.49± 3.35 a	7.31± 2.82 a
LDL-3 %free chol	7.46± 0.27	6.96± 0.33 a	7.04± 0.37 a	9.15± 0.62	8.05± 0.68 b	8.02± 0.65 b
LDL-3 %est. chol	40.4± 0.58	41.6± 0.70 a	41.4± 0.58 a	36.9± 1.72	38.2± 1.84	38.8± 1.83
%VLDL-1/ ΣVLDL	31.0± 3.37	41.7± 2.62 c	42.9± 3.68 c	22.5± 2.58	34.1± 1.79 b	29.8± 2.61 a
%VLDL-2/ ΣVLDL	28.0± 0.81	28.7± 0.50	30.0± 0.79	24.8± 1.96	27.6± 1.87	27.5± 1.79
%VLDL-3/ ΣVLDL	41.1± 3.54	29.6± 2.65 c	27.1± 3.56 c	52.7± 2.83	38.4± 3.16 b	42.8± 3.89 a
%LDL-3/ ΣLDL	45.1± 2.20	45.1± 2.17	44.1± 2.54	23.7± 3.60	24.2± 3.22	23.3± 2.99
LDL chol/ HDL chol	3.52± 0.37	3.45± 0.34	3.87± 0.43	1.89± 0.34	1.96± 0.35	1.98± 0.36 a
HDL_2/ HDL_3 chol	0.60± 0.09	0.67± 0.10	0.70± 0.15	0.99± 0.26	0.88± 0.17	0.93± 0.19
HDL trig/chol	0.28± 0.03	0.30± 0.05	0.39± 0.06	0.12± 0.02	0.16± 0.03 a	0.17± 0.02 a
HDL_2 trig/chol	0.21± 0.03	0.28± 0.06	0.32± 0.04	0.10± 0.03	0.15± 0.03 a	0.14± 0.02 a
HDL_3 trig/chol	0.38± 0.08	0.34± 0.05	0.45± 0.05	0.15± 0.02	0.18± 0.03	0.19± 0.04
Glucose (mM)	10.1± 0.67	13.6± 1.13 c	10.2± 0.97	5.49± 0.10	6.15± 0.24 a	5.37± 0.14
Insulin (mU/l)	11.9± 3.35	102± 42.7 c	26.1± 5.52 b	9.21± 1.44	42.3± 10.1 b	10.1± 1.75
NEFA (mM)	0.81± 0.06	0.61± 0.08	1.06± 0.14	0.57± 0.07	0.48± 0.05	0.69± 0.08

All values are mean ± SEM.
chol: cholesterol; trig: triglyceride.
Fasting levels vs levels at 3 and 8 hours. a: $p<0.05$; b: $p<0.01$; c $p<0.001$

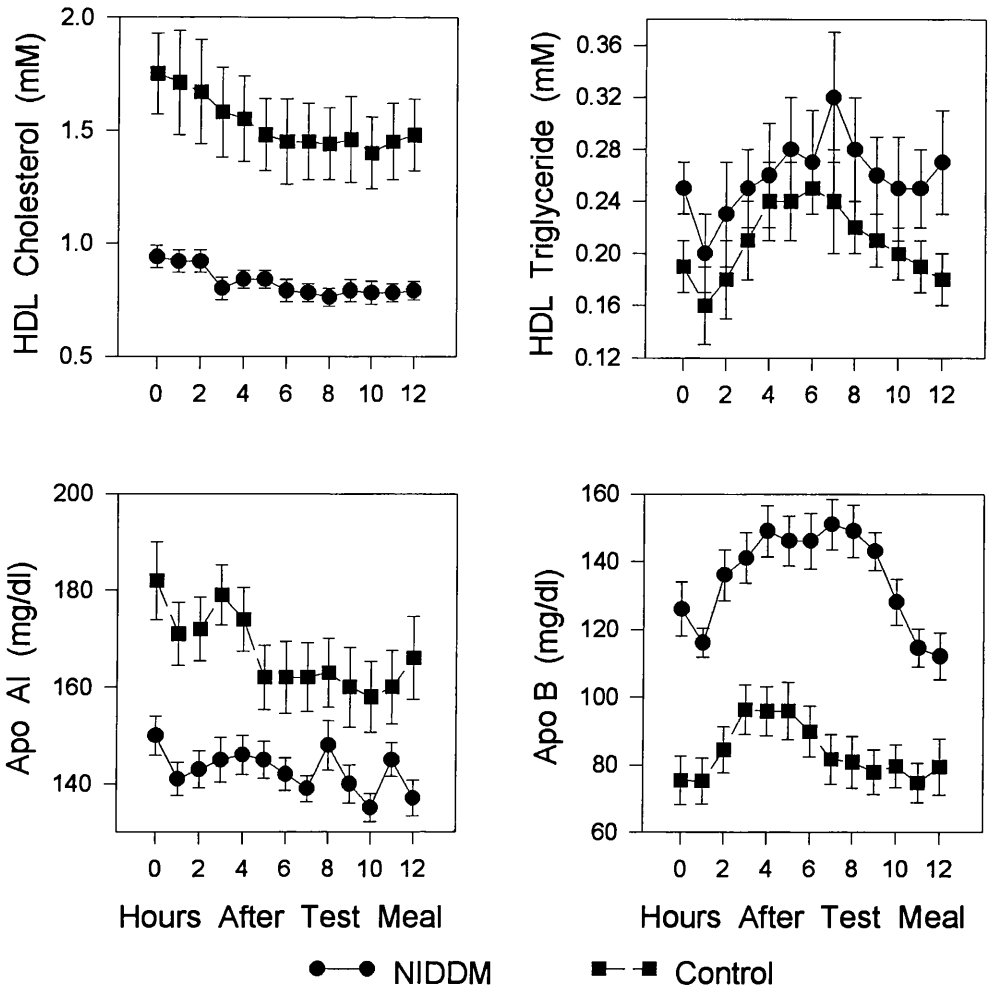


Fig. 3.3. Fasting and postprandial concentrations of HDL cholesterol and triglyceride, apo AI and apo B.

Table 3.9. AUC and IAUC in diabetic patients and controls.

	NIDDM AUC	Control AUC	NIDDM IAUC	Control IAUC
HDL cholesterol (mM.hrs)	9.93±0.49	18.3±2.18 b	-1.3±0.21	-2.73±0.86
HDL Triglyceride (mM.hrs)	3.10±0.39	2.52±0.25	0.12±0.27	0.25±0.18
Apo AI (mg/dl.hrs)	1691±41	1995±82 b	-103±44.0	-191±33.3
Apo B (mg/dl.hrs)	1640±57	1009±77 c	124±68.2	105±26.9

All areas were calculated as a product of concentration (units as in table above) and time (hours) over 12 hours. All values are mean ± SEM. b: $p<0.01$; c: $p<0.001$

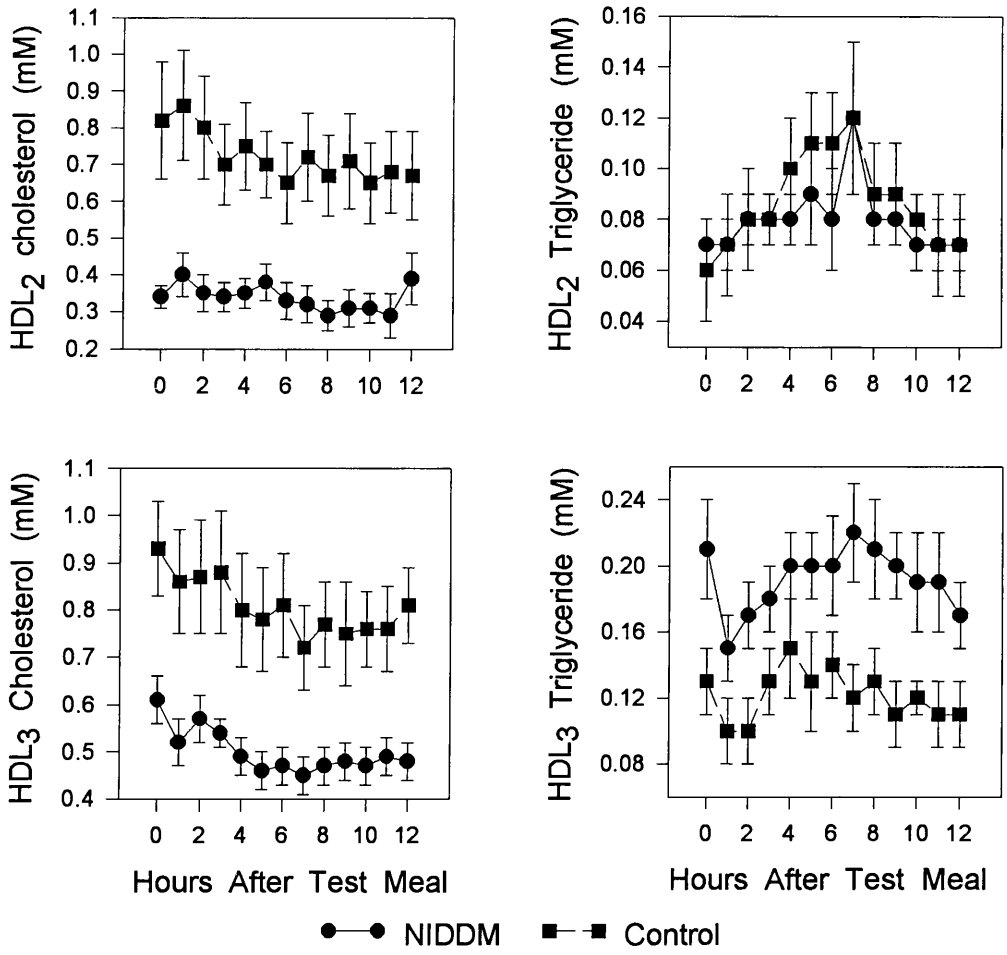


Fig. 3.4. Fasting and postprandial concentrations of HDL_2 cholesterol and triglyceride and HDL_3 cholesterol and triglyceride.

Table 3.10. AUC and IAUC in diabetic patients and controls.

	NIDDM AUC	Control AUC	NIDDM IAUC	Control IAUC
HDL_2 cholesterol (mM.hrs)	4.04±0.53	8.62±1.35 b	0.02±0.36	-1.24±0.92
HDL_2 triglyceride (mM.hrs)	0.98±0.14	1.08±0.18	0.13±0.19	0.31±0.13
HDL_3 cholesterol (mM.hrs)	5.95±0.51	9.63±1.20 b	-1.33±0.42	-1.52±0.32
HDL_3 triglyceride (mM.hrs)	2.29±0.26	1.46±0.18 a	-0.24±0.27	-0.06±0.20

All areas were calculated as a product of concentration (units as in table above) and time (hours) over 12 hours. All values are mean ± SEM. a: $p<0.05$; b: $p<0.01$

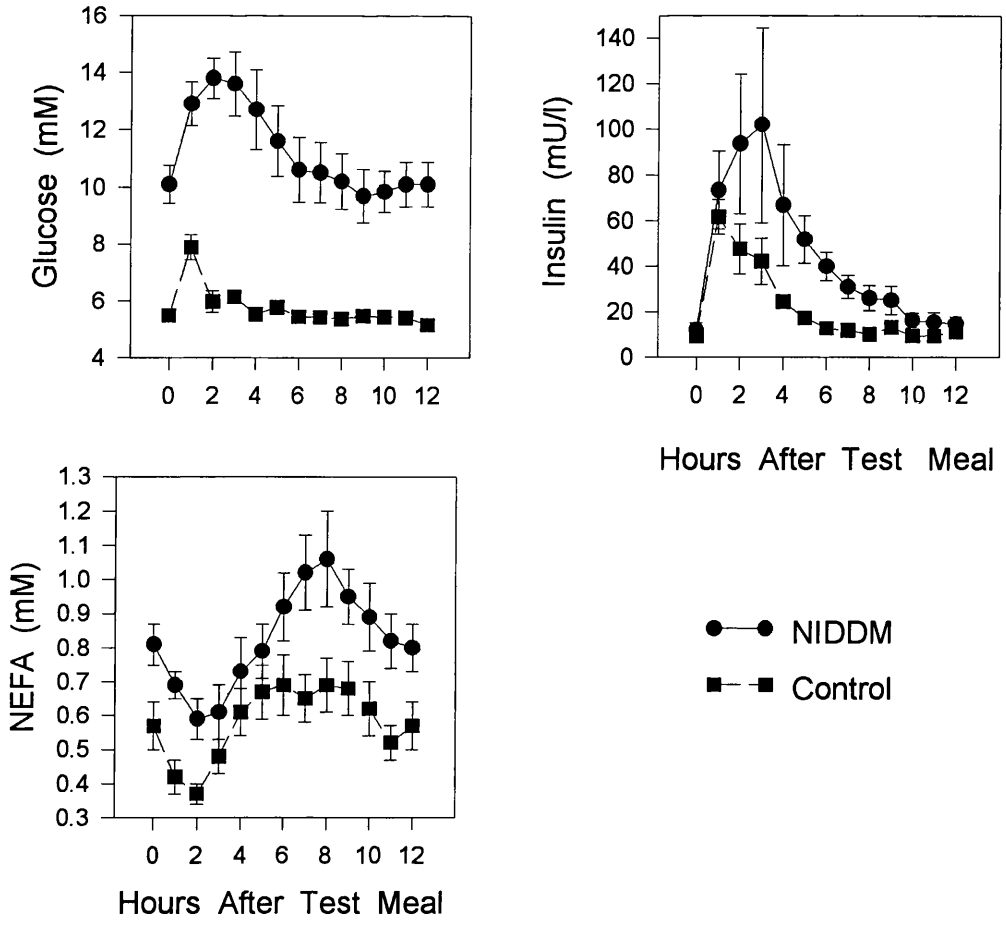


Fig. 3.5 Fasting and postprandial concentrations of glucose, total insulin and NEFA.

Table 3.11. AUC and IAUC in diabetic patients and controls.

	NIDDM AUC	Control AUC	NIDDM IAUC	Control IAUC
Glucose (mM.hrs)	139±9.69	69.2±1.19 c	17.6±4.12	3.32±1.20 b
Total insulin (mU/l.hrs)	555±150	270±31.5 a	412±122	160±25.3
NEFA (mM.hrs)	9.89±0.67	6.99±0.51 b	0.20±0.86	0.14±0.66

All areas were calculated as a product of concentration (units as in table above) and time (hours) over 12 hours. All values are mean ± SEM. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

3.3.4. Postprandial chylomicrons, VLDL subfractions and LDL subfractions in diabetic patients and controls

Postprandial response for concentration (mass) of chylomicrons, VLDL subfractions and LDL subfractions are shown in Figs. 3.6 and 3.7 and AUC and IAUC for these data are shown underneath in Tables 3.12 and 3.13 respectively. Comparisons between fasting levels and levels at 3 and 8 hours for these parameters within each subject group are also shown in these figures. Comparisons between diabetic patients and controls of the levels of the lipid and protein components of chylomicrons, VLDL subfractions and LDL subfractions at 3 and at 8 hours are shown in Tables 3.15 and 3.17 respectively.

After the test meal, levels of chylomicrons, VLDL-1 and -2 increased in both subject groups (Fig. 3.6), and the AUC for these lipoprotein fractions were significantly elevated in diabetic patients compared to controls (Table 3.12). For chylomicrons and VLDL-1, in both subject groups, concentrations were significantly higher at 3 and 8 hours compared to fasting levels (Fig. 3.6). However, whereas in diabetic patients the concentrations of chylomicrons and VLDL-1 continued to increase up to 8 hours, in controls the concentrations were less at 8 hours than at 3 hours. This was particularly evident in the VLDL-1 fraction, since diabetic patients had significantly raised IAUC compared to controls (Table 3.12). VLDL-2 increase was higher in controls as shown by the comparison of concentrations at 3 and 8 hours with fasting levels in diabetic patients and controls (Fig. 3.6). Despite diabetic patients having significantly higher AUC for VLDL-3 (Table 3.12), their levels of VLDL-3 fell after the test meal, whereas in controls levels increased (Fig. 3.6). This difference is confirmed by the comparison of the IAUC, where controls had significantly raised IAUC for VLDL-3 compared to diabetic patients (Table 3.12). In addition, there was a significant reduction in VLDL-3 after 8 hours in diabetic patients, whereas in controls the levels were clearly elevated after 8 hours, although the difference did not reach significance. The AUC and IAUC for triglyceride in chylomicrons and VLDL subfractions showed similar trends to that for total mass in diabetic patients and controls (Table 3.14).

All LDL subfractions decreased after the test meal in both subject groups (Fig. 3.7), and elevated fasting levels of LDL-3 persisted postprandially, as shown by the significantly raised AUC for LDL-3 in diabetic patients compared to controls (Table 3.13). Reductions in LDL-1 and LDL-2 were similar in patients and controls, since there were no differences in IAUC (Table 3.13) or paired comparisons within each subject group for these subfractions (Fig. 3.7). As shown by analysis of concentrations of LDL-3 at 3 and 8 hours

compared to fasting levels, there was a more significant decrease in LDL-3 in diabetic patients after the test meal compared to the control group (Fig. 3.7). The AUC and IAUC for cholesterol in LDL subfractions showed similar trends to that for total LDL mass in diabetic patients and controls (Table 3.14). Similarly to fasting comparisons, there were no differences in postprandial total LDL cholesterol, but diabetic patients had significantly higher LDL triglyceride (Table 3.14). As shown by the negative IAUC values, total LDL cholesterol and triglyceride decreased after the test meal in both subject groups.

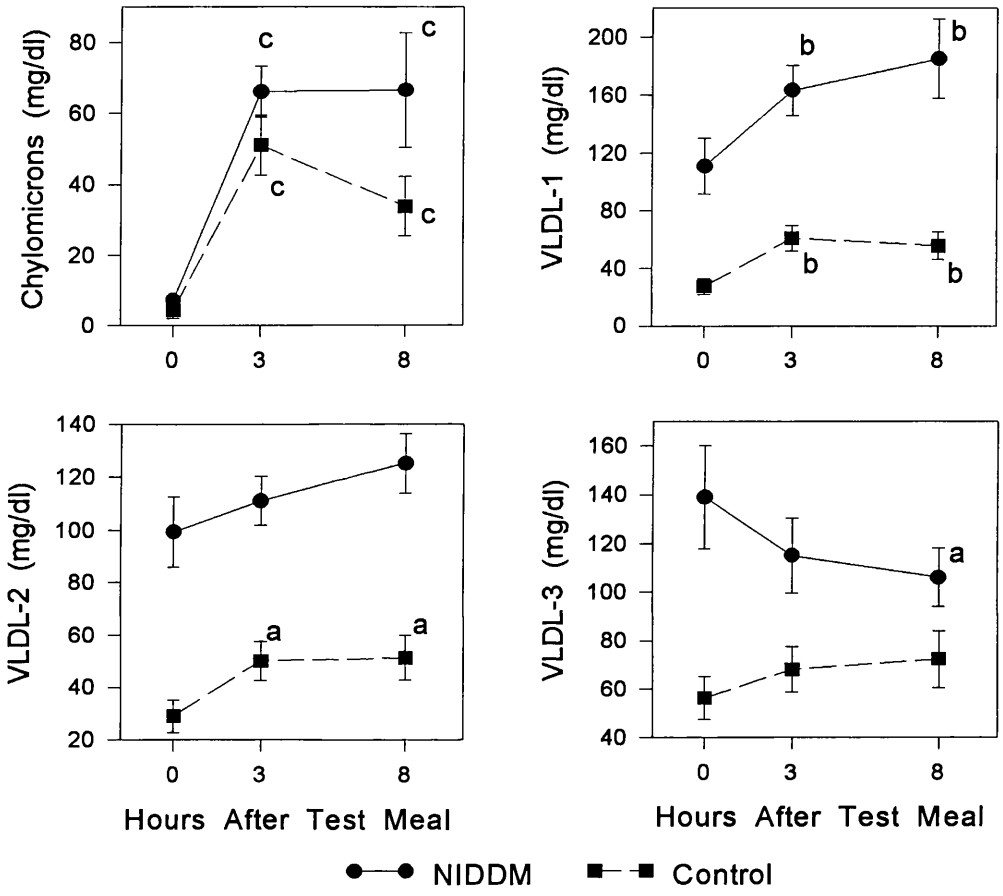


Fig. 3.6. Fasting and postprandial concentrations of chylomicrons, VLDL-1, -2, and -3. For comparisons of paired data (0hrs vs 3hrs and 0hrs vs 8hrs) within each subject group: a: $p<0.05$; b: $p<0.01$; c: $p<0.001$.

Table 3.12. AUC and IAUC in diabetic patients and controls.

	NIDDM AUC	Control AUC	NIDDM IAUC	Control IAUC
Chylomicron mass (mg/dl.hrs)	442±47.4	295±45.5 a	384±46.7	261±46.9
VLDL-1 mass (mg/dl.hrs)	1278±158	424±59.5 c	392±56.3	204±27.0 b
VLDL-2 mass (mg/dl.hrs)	905±74.5	372±53.0 c	112±61.6	140±43.2
VLDL-3 mass (mg/dl.hrs)	933±119	538±76.7 b	-178±76.0	88.2±47.6 b

All areas were calculated as a product of concentration (units as in table above) and time (hours) over 8 hours. All values are mean ± SEM. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

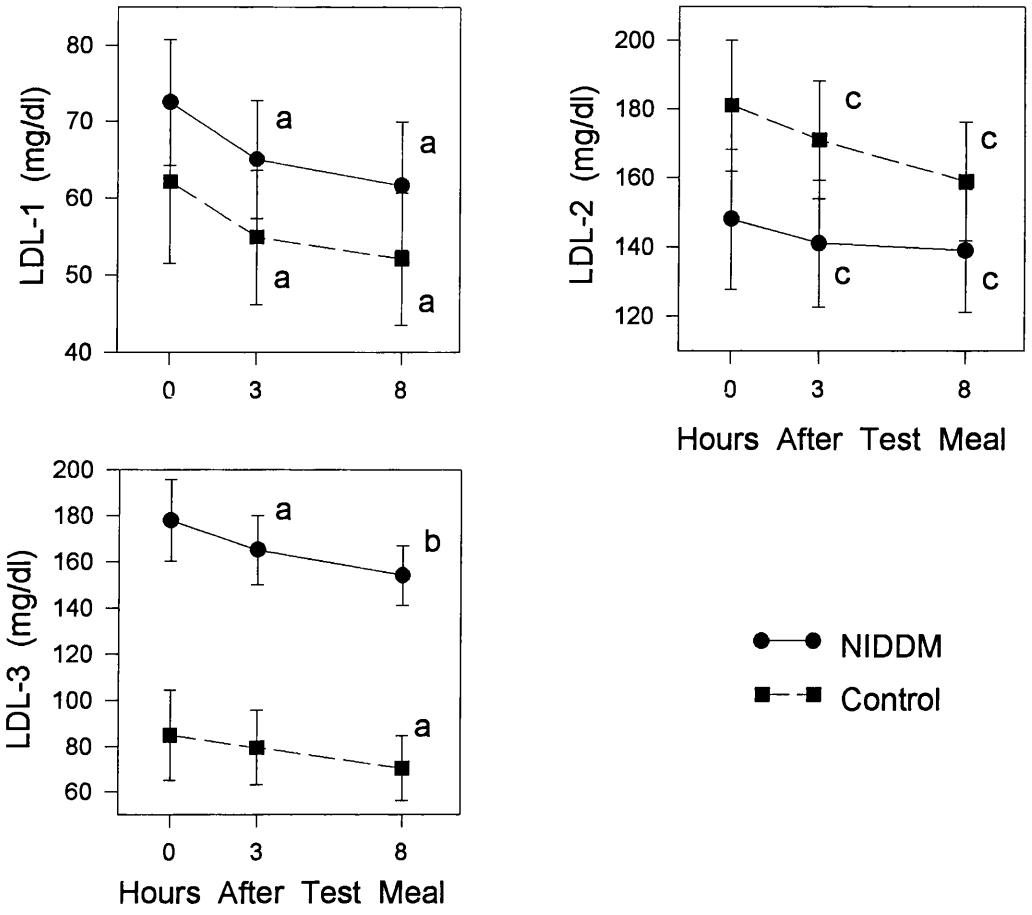


Fig. 3.7. Fasting and postprandial concentrations of LDL-1, -2 and -3.

For comparisons of paired data (0hrs vs 3hrs and 0hrs vs 8hrs) within each subject group: a: $p<0.05$; b: $p<0.01$; c: $p<0.001$.

Table 3.13. AUC and IAUC in diabetic patients and controls.

	NIDDM AUC	Control AUC	NIDDM IAUC	Control IAUC
LDL-1 mass (mg/dl.hrs)	523±62.2	443±71.1	-56.9±28.0	-54.0±21.9
LDL-2 mass (mg/dl.hrs)	1134±145	1353±140	-50.7±38.4	-92.0±24.8
LDL-3 mass (mg/dl.hrs)	1311±118	621±130 c	-112±35.0	-57.9±35.0

All areas were calculated as a product of concentration (units as in table above) and time (hours) over 8 hours. All values are mean ± SEM. c: $p<0.001$

Table 3.14. AUC and IAUC for chylomicrons and VLDL and LDL subfractions in diabetic patients and controls.

	NIDDM AUC	Control AUC	NIDDM IAUC	Control IAUC
Chylomicron chol	16.3±2.15	12.4±2.56	11.2±2.54	8.42±1.39
VLDL-1 chol	88.9±11.9	38.5±7.20 b	22.1±5.61	15.0±2.35
VLDL-2 chol	96.8±10.9	42.5±6.02 c	6.50±9.83	14.4±4.20
VLDL-3 chol	161±25.4	99.2±19.6	-45.2±17.8	8.60±9.55 a
LDL-1 chol	153±21.1	132±23.6	-18.9±12.0	-20.1±6.64
LDL-2 chol	362±53.3	456±50.3	-24.1±14.9	-36.8±7.85
LDL-3 chol	417±40.2	198±41.0 b	-32.3±9.72	-19.9±11.1
Total LDL chol	932±93.6	785±83.2	-75.3±30.7	-76.8±13.8
Chylomicron trig	363±40.8	233±38.1 a	321±39.3	215±39.2
VLDL-1 trig	857±113	272±40.4 c	267±41.3	133±16.9 b
VLDL-2 trig	513±40.0	204±32.4 c	74.3±33.7	77.6±28.1
VLDL-3 trig	382±42.7	213±25.2 b	-59.0±35.4	47.9±24.3 a
LDL-1 trig	81.8±8.37	65.3±8.68	-13.1±4.46	-7.98±5.17
LDL-2 trig	78.0±11.0	61.9±6.99	-3.26±3.50	-1.91±1.47
LDL-3 trig	57.5±5.15	29.6±5.92 b	-0.54±2.34	1.72±1.26
Total LDL trig	217±22.0	157±16.3 a	-16.9±8.64	-8.17±6.49

All areas were calculated as a product of concentration (mg/dl) and time (hours) over 8 hours. All values are mean ± SEM.
chol: cholesterol; trig: triglyceride. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Results from analyses of composition (expressed as percentage of total mass) of chylomicrons, VLDL subfractions and LDL subfractions at 3 and 8 hours are shown in Tables 3.16 and 3.18 respectively. At 3 hours after the test meal there was a significant increase in % triglyceride and decrease in % free cholesterol in chylomicrons and LDL-2 in diabetic patients compared to controls. In addition, % esterified cholesterol in VLDL-1 was significantly reduced in diabetic patients. At 8 hours after the test meal there was a significant increase in % triglyceride and decrease in % free cholesterol in LDL-2 in diabetic patients compared to controls.

Paired analyses of composition of chylomicrons, VLDL subfractions and LDL subfractions at 3 and 8 hours compared to fasting values are shown in Table 3.8. There was a significant reduction in postprandial % free cholesterol in VLDL-2, VLDL-3, LDL-1, LDL-2 and LDL-3 in controls, and in VLDL-1 and LDL-3 in diabetics patients compared to fasting levels. In controls only, postprandial % triglyceride in VLDL-3 and LDL-2 was significantly elevated. A significant increase in postprandial % triglyceride occured in LDL-3 for both subject groups. In addition, postprandial % esterified cholesterol in LDL-3 was significantly elevated in diabetic patients compared to fasting levels.

Table 3.15. Concentrations of lipid and protein components of chylomicrons, VLDL and LDL subfractions 3 hours after test meal.

	Chylomicrons		VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr
CH	2.15± 0.29	2.04± 0.32	10.9± 1.30	5.25± 0.81 b	11.7± 1.33	5.52± 0.68 c	19.8± 3.02	12.4± 2.47	19.1± 2.63	16.2± 2.90	44.6± 6.66	57.1± 6.21	52.7± 5.28	25.2± 5.18 b
TG	55.1± 5.84	40.7± 7.08	110± 12.5	39.7± 6.33 c	63.2± 5.12	27.9± 4.85 c	46.9± 6.18	27.4± 3.09 a	9.94± 1.01	7.98± 0.96	9.71± 1.29	7.53± 0.84	7.28± 0.65	3.84± 0.77 b
PL	3.63± 0.67	3.37± 0.50	20.7± 2.71	8.64± 1.55 b	17.8± 2.01	8.75± 1.54 b	22.3± 3.12	13.6± 2.10 a	13.8± 1.53	11.8± 2.06	30.5± 4.15	35.9± 2.96	31.4± 3.19	15.8± 3.47 a
FC	1.08± 0.18	1.34± 0.25	6.81± 0.65	3.11± 0.49 c	6.35± 0.51	2.91± 0.48 c	8.85± 1.02	5.88± 1.17	5.95± 0.66	5.54± 1.08	12.2± 1.51	16.4± 1.59	11.5± 1.18	6.57± 1.35 a
EC	1.80± 0.25	1.34± 0.25	6.84± 1.19	3.59± 0.58 a	8.90± 1.48	4.38± 0.49 a	18.4± 3.46	11.0± 2.23	22.1± 3.53	18.0± 3.09	54.5± 8.81	68.4± 7.96	69.2± 7.06	31.4± 6.48 b
Prot	4.50± 0.85	4.42± 0.95	18.7± 1.72	5.90± 0.72 c	14.8± 1.38	6.13± 0.76 c	18.7± 2.66	10.3± 1.35 a	13.3± 1.91	11.6± 1.80	34.2± 3.29	43.0± 5.21	45.6± 3.51	21.8± 4.69 b
Mass	66.2± 7.11	51.1± 8.47	163± 17.5	60.9± 8.82 c	111± 9.23	50.1± 7.62 c	115± 15.4	68.1± 9.50 a	65.0± 7.71	54.9± 8.70	141± 18.3	171± 17.1	165± 15.0	79.4± 16.3 b

All concentrations are mean ± SEM (mg/dl).

NIDD: NIDDM; Contr: Controls; CH: cholesterol; TG: triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Table 3.16. Composition of chylomicrons, VLDL and LDL subfractions 3 hours after test meal.

	Chylomicrons		VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr
CH	3.24± 0.26	4.39± 0.63	6.67± 0.32	8.94± 0.98 a	10.3± 0.46	11.9± 1.29	17.0± 0.54	17.2± 1.10	29.0± 0.83	28.7± 1.19	31.0± 1.00	33.3± 0.83	31.7± 0.39	30.8± 1.44
TG	83.6± 1.29	78.6± 1.45 a	66.9± 0.92	64.7± 2.07	57.1± 1.50	54.7± 3.02	40.0± 2.41	41.9± 1.65	15.8± 1.03	15.3± 1.63	7.11± 0.49	4.42± 0.40 c	4.46± 0.26	7.49± 3.35
PL	5.57± 0.71	6.74± 0.40	12.5± 0.71	13.4± 0.81	15.8± 0.91	16.5± 1.50	20.2± 2.03	19.6± 0.74	21.4± 0.49	21.3± 0.66	21.5± 0.62	21.3± 0.66	19.0± 0.54	19.3± 0.88
FC	1.59± 0.19	2.61± 0.32 a	4.26± 0.12	5.29± 0.61	5.79± 0.28	6.22± 1.04	8.06± 0.63	8.39± 0.53	9.27± 0.39	9.51± 0.68	8.76± 0.29	9.65± 0.30 a	6.96± 0.33	8.05± 0.68
EC	2.78± 0.27	2.98± 0.57	4.05± 0.57	6.12± 0.71 a	7.62± 0.76	9.60± 0.97	15.0± 1.39	14.7± 1.26	33.2± 1.94	32.3± 1.76	37.3± 2.08	39.7± 1.38	41.6± 0.70	38.2± 1.84
Prot	6.47± 0.90	9.07± 1.09	12.3± 1.57	10.5± 1.12	13.7± 1.31	13.0± 0.96	16.7± 1.47	15.3± 0.61	20.3 ± 1.56	21.6± 0.91	25.3± 1.73	24.9± 1.21	28.0± 0.87	26.9± 0.92

All values are mean % of total mass ± SEM.

NIDD: NIDDM; Contr: Controls; CH: cholesterol; TG: triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Table 3.17. Concentrations of lipid and protein components of chylomicrons, VLDL and LDL subfractions 8 hours after test meal.

	Chylomicrons		VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr
CH	2.71± 0.57	1.40± 0.40	13.1± 2.09	5.23± 1.18 b	13.3± 1.49	6.07± 1.18 b	17.3± 2.66	13.0± 2.95	17.8± 2.85	15.3± 2.73	44.4± 6.39	54.0± 6.35	48.8± 4.40	22.5± 4.49 c
TG	53.7± 14.1	26.8± 7.42	123± 19.7	35.0± 5.85 c	71.1± 7.04	27.6± 4.63 b	44.6± 4.48	29.2± 3.79 b	9.69± 1.15	7.85± 1.22	9.56± 1.56	7.94± 0.94	7.0± 0.60	3.61± 0.71 b
PL	4.91± 1.42	2.27± 0.47	22.8± 3.46	7.89± 1.41 c	19.9± 1.67	9.09± 1.63 c	20.7± 2.17	14.4± 2.60	13.1± 1.62	10.9± 1.97	28.7± 3.75	33.5± 3.23	29.5± 2.77	14.0± 3.07 b
FC	1.44± 0.55	0.62± 0.20	7.30± 1.44	2.95± 0.77 a	6.50± 0.51	2.78± 0.70 c	7.59± 0.78	6.05± 1.36	5.78± 0.69	5.02± 0.91	11.1± 1.35	15.3± 1.65	10.7± 0.98	5.88± 1.23 b
EC	2.13± 0.34	1.31± 0.35	9.79± 1.87	3.83± 0.73 b	11.4± 1.90	5.53± 1.18 b	16.2± 3.26	11.7± 2.69	20.3± 3.80	17.2± 3.11	55.9± 8.64	65.1± 8.05	63.9± 5.97	27.9± 5.54 c
Prot	4.36± 0.99	2.76± 0.68	21.7± 2.93	6.09± 1.38 c	15.8± 1.67	6.43± 1.13 c	16.4± 2.04	11.1± 1.76	12.8± 1.70	11.1± 1.70	34.5± 3.59	37.3± 4.23	42.3± 3.40	19.0± 4.13 c
Mass	66.6± 16.2	33.8± 8.47	185± 27.3	55.8± 9.64 c	125± 11.3	51.4± 8.64 b	106± 12.1	72.4± 11.8 b	61.6± 8.38	52.1± 8.59	140± 17.8	159± 17.2	154± 12.9	70.4± 14.3 b

All concentrations are mean ± SEM (mg/dl).

NIDD: NIDDM; Contr: Controls; CH: cholesterol; TG: triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Table 3.18. Composition of chylomicrons, VLDL and LDL subfractions 8 hours after test meal.

	Chylomicrons		VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr	NIDD	Contr
CH	4.91± 0.99	4.85± 1.05	7.00± 0.28	9.27± 0.83 a	10.5± 0.36	11.4± 0.72	15.9± 0.58	17.2± 0.84	28.5± 0.70	28.8± 1.06	31.3± 0.86	33.8± 0.66 a	31.7± 0.33	31.1± 1.38
TG	75.7± 6.80	76.3± 2.48	65.7± 1.20	62.4± 2.30	56.6± 1.30	53.9± 1.43	42.8± 1.41	41.8± 1.32	16.2± 0.96	15.2± 1.33	6.99± 0.65	5.00± 0.45 a	4.62± 0.30	7.31± 2.82
PL	7.50± 1.38	7.77± 1.29	12.6± 0.54	14.9± 0.99	16.1± 0.44	17.9± 0.76	19.8± 0.67	19.3± 0.69	21.5± 0.61	20.9± 0.69	20.6± 0.43	21.3± 0.63	19.3± 0.73	19.4± 0.74
FC	2.01± 0.39	1.94± 0.49	3.86± 0.24	4.79± 0.59	5.32± 0.26	4.95± 0.84	7.31± 0.29	8.04± 0.40	9.58± 0.53	9.47± 0.47	8.17± 0.42	9.67± 0.32 a	7.04± 0.37	8.02± 0.65
EC	4.86± 1.23	4.89± 1.08	5.26± 0.69	7.53± 1.06	8.73± 0.91	10.8± 0.81	14.4± 1.23	15.3± 0.86	31.7± 1.72	32.5± 2.03	38.9± 1.70	40.6± 1.11	41.4± 0.58	38.8± 1.83
Prot	9.91± 4.24	9.09± 1.23	12.6± 1.64	10.5± 0.87	13.3± 1.65	12.5± 0.79	15.6± 1.19	15.6± 0.55	21.0± 1.43	22.0± 1.27	25.4± 1.89	23.4± 0.65	27.6± 0.90	26.5± 1.03

All values are mean % of total mass ± SEM.

NIDD: NIDDM; Contr: Controls; CH: cholesterol; TG: triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Comparisons of qualitative parameters at 3 and 8 hours are shown in Table 3.19. In general, differences in these qualitative parameters between diabetic patients and controls are similar at 3 and 8 hours to those in the fasting state. Percent VLDL-1/ΣVLDL was significantly higher at 3 and 8 hours in diabetic patients than in controls, and %VLDL-3/ΣVLDL was significantly reduced at 8 hours in diabetic patients. There were no differences in % VLDL-2/ΣVLDL between diabetic patients and controls. Diabetic patients had significantly elevated % LDL-3/ΣLDL and LDL/HDL cholesterol ratio at 3 and 8 hours compared to controls. HDL_2/HDL_3 cholesterol ratio was reduced at 3 and 8 hours by 14% and 15% respectively in diabetic patients compared to controls, although results did not reach significance. HDL, HDL_2 and HDL_3 was triglyceride enriched at 3 and 8 hours in diabetic patients (not significant for HDL_3 at 3 hours), as shown by triglyceride/cholesterol ratios for these lipoproteins.

Paired analyses between qualitative parameters at 3 and 8 hours compared to fasting values for both subject groups is shown in Table 3.8. After the test meal, %VLDL-1/ΣVLDL significantly increased, % VLDL-3/ΣVLDL significantly decreased and % VLDL-2/ΣVLDL, %LDL-3/ΣLDL and HDL_2/HDL_3 cholesterol ratio remained the same for both diabetic patients and controls. LDL/HDL cholesterol ratio was significantly elevated at 8 hours in the control group only. Triglyceride/cholesterol ratio for HDL, HDL_2 and HDL_3 was elevated postprandially in both subject groups, but only reaching significance for HDL and HDL_2 in controls.

Table 3.19. Qualitative parameters in diabetic patients and controls 3 and 8 hours after test meal.

	3 hours		8 hours	
	NIDDM	Control	NIDDM	Control
%VLDL-1/ ΣVLDL	41.7±2.62	34.1±1.79 a	42.9±3.68	29.8±2.61 a
%VLDL-2/ ΣVLDL	28.7±0.50	27.6±1.87	30.0±0.79	27.5±1.79
%VLDL-3/ ΣVLDL	29.6±2.65	38.4±3.16	27.1±3.56	42.8±3.89 b
%LDL-3/ΣLDL	45.1±2.17	23.7±3.60 c	44.1±2.54	24.2±3.22 c
LDL chol/ HDL chol	3.45±0.34	1.96±0.35 b	3.87±0.43	1.98±0.36 b
HDL_2/ HDL_3 chol	0.67±0.10	0.88±0.17	0.70±0.15	0.93±0.18
HDL trig/chol	0.30±0.05	0.16±0.03 a	0.39±0.06	0.17±0.02 b
HDL_2 trig/chol	0.28±0.06	0.15±0.03 a	0.31±0.04	0.14±0.02 c
HDL_3 trig/chol	0.34±0.05	0.18±0.03	0.45±0.05	0.19±0.04 b

All values are mean ± SEM.
chol: cholesterol; trig: triglyceride
a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

3.3.5. Lipids, lipoproteins, lipoprotein subfractions and metabolites: their relationship with postprandial triglyceride and small dense LDL

Correlations of BMI, fasting values of lipids, lipoproteins, lipoprotein subfractions and metabolites with postprandial triglyceride response (AUC for serum triglyceride) are shown in Table 3.20. Correlations were made in three subject groups: i) 10 diabetic patients; ii) 10 non-diabetic controls; iii) in 18 diabetic patients (studied in chapter 4, which include the 10 diabetic patients in this chapter, and which have similar entry criteria).

The parameters which had the strongest association with postprandial triglyceride were serum triglyceride and triglyceride-rich lipoproteins. BMI and fasting values of triglyceride for serum and triglyceride-rich lipoproteins were positively associated with postprandial triglyceride in all groups. More of these correlations were significant in the control group than in the 10 diabetic patients. However, highly significant correlations in the 18 diabetic patients (probably due to larger patient number) suggest that these associations in the 10 diabetic patients are reliable. There were neither significant correlations with HDL, HDL_2 and HDL_3 cholesterol nor with any of the metabolites. However there were non-significant positive associations with insulin resistance in all groups.

Table 3.20. Correlation coefficients (r) for lipids, lipoproteins and metabolites with postprandial triglyceride.

	AUC Triglyceride		
	NIDDM (n=10)	Control (n=10)	NIDDM (n=18)
BMI	0.38	0.74 a	0.34
Triglyceride	0.62	0.86 b	0.89 c
VLDL trig	0.67 a	0.66 a	0.90 c
Chylomicron trig	0.51	0.64 a	0.71 c
VLDL-1 trig	0.74 a	0.87 b	0.84 c
VLDL-2 trig	0.56	0.51	0.89 c
VLDL-3 trig	0.26	0.53	0.76 c
HDL chol	-0.05	-0.19	-0.28
HDL_2 chol	-0.42	-0.30	0.06
HDL_3 chol	0.04	0.03	-0.40
NEFA	0.40	-0.12	-0.05
Total Insulin	0.38	0.16	0.24
Glucose	-0.02	0.57	-0.37
Insulin resistance	0.37	0.23	0.37
β-cell function %	0.36	-0.44	-0.08

Values are Pearson's correlation coefficients for the 10 diabetic and 10 control subjects used in this chapter, and additionally the 18 diabetics studied in chapter 4. These 18 diabetics include the 10 subjects from this chapter, and have the same entry criteria.
a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Correlations of BMI, and fasting and AUC values lipids, lipoproteins, lipoprotein subfractions and metabolites with the proportion of small, dense LDL (%LDL-3/ΣLDL) are shown in Table 3.21. Correlations were made in three subject groups: i) 10 diabetic patients; ii) 10 non-diabetic controls; iii) in 18 diabetic patients (studied in chapter 4, which include the 10 diabetic patients in this chapter, and which have similar entry criteria).

The strongest correlations with %LDL-3/ΣLDL involved triglyceride-rich lipoproteins and HDL cholesterol. BMI, serum triglyceride and triglyceride-rich lipoproteins were positively associated with %LDL-3/ΣLDL in diabetic patients, the majority of these correlations reaching significance for the 18 diabetic patients. Similar trends can be seen for controls, with the exception of negative associations with chylomicrons and proportion of large VLDL (VLDL-1/ΣVLDL). In the 18 diabetic patients and controls correlations of %LDL-3/ΣLDL with triglyceride-rich lipoproteins were weaker with AUC values than with fasting values. %LDL-3/ΣLDL correlated negatively with fasting and AUC values for HDL and HDL_2 cholesterol in all groups, reaching significance for controls and the 18 diabetic patients. There were no significant

correlations with HDL_3 cholesterol. Fasting and postprandial NEFA correlated positively and significantly with %LDL-3/ΣLDL in diabetic patients, and there was also a significant positive correlation with fasting glucose in the 18 diabetic patients. However, since fasting NEFA did not correlate significantly with %LDL-3/ΣLDL in the 18 diabetic patients this result may not be reliable. Insulin resistance was positively associated with %LDL-3/ΣLDL in diabetic patients (p=0.06 for the 18 diabetic patients).

Table 3.21. Correlation coefficients (r) of %LDL-3/ΣLDL mass with fasting and postprandial levels of lipids, lipoproteins and metabolites.

	NIDDM (n=10)	Control (n=10)	NIDDM (n=18)
BMI	0.13	0.17	0.54 a
Triglyceride	0.54	0.30	0.74 c
Triglyceride AUC	0.37	0.12	0.52 a
VLDL trig	0.55	0.54	0.75 c
VLDL trig AUC	0.62	0.39	0.61 b
Chylo trig	0.39	-0.32	0.56 a
Chylo trig AUC	0.62	-0.16	0.25
VLDL-1 trig	0.58	0.38	0.69 b
VLDL-1 trig AUC	0.32	0.37	0.64 b
VLDL-2 trig	0.51	0.61	0.74 c
VLDL-2 trig AUC	0.11	0.52	0.68 b
VLDL-3 trig	0.25	0.56	0.69 b
VLDL-3 trig AUC	-0.23	0.35	0.35
%VLDL-1/ΣVLDL	0.48	-0.06	0.56 a
HDL chol	-0.41	-0.89 c	-0.64 b
HDL chol AUC	-0.38	-0.72 a	-0.57 a
HDL_2 chol	-0.60	-0.82 b	-0.55 a
HDL_2 chol AUC	-0.73 a	-0.79 b	-0.60 b
HDL_3 chol	-0.37	-0.32	-0.22
HDL_3 chol AUC	0.47	-0.43	-0.12
NEFA	0.84 b	0.01	0.21
NEFA AUC	0.33	0.49	0.61 b
Insulin	0.45	-0.11	0.28
Insulin AUC	0.06	0.46	0.19
Glucose	0.10	0.32	0.52 a
Glucose AUC	0.02	0.13	0.35
Insulin resistance	0.50	-0.08	0.45
β-cell function %	0.39	-0.18	-0.12

Values are Pearson's correlation coefficients for the 10 diabetic and 10 control subjects used in this chapter, and additionally the 18 diabetics studied in chapter 4. These 18 diabetics include the 10 subjects from this chapter, and have the same entry criteria.
a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

To assess the combined influence of serum triglyceride, HDL cholesterol and insulin resistance on %LDL-3/ΣLDL in patients with NIDDM, multivariate analysis was performed with the 18 diabetic patients with %LDL-3/ΣLDL as the dependent variable. Two models were constructed, one with fasting lipid values (Table 3.22) and the other with postprandial (AUC) values (Table 3.23).

Fasting triglyceride was significantly associated with %LDL-3/ΣLDL, whereas association with HDL cholesterol did not quite reach statistical significance. Insulin resistance was less strongly associated with %LDL-3/ΣLDL than the lipid values indicating that the lipid values, particularly triglyceride, had more influence on the variability of %LDL-3/ΣLDL. In this way, the outcome of multivariate analysis agrees with that of univariate analysis in which the strength of association for insulin resistance was also less than that for these lipid values.

Postprandial lipid values were less strongly associated with %LDL-3/ΣLDL. This is consistent with univariate analysis findings which show that these postprandial lipid values have weaker associations with %LDL-3/ΣLDL than the fasting values.

Table 3.22. Multiple regression analysis for the determinants of %LDL-3/ΣLDL mass ratio in the fasting state in 18 diabetics

	Association	p value
Triglyceride	positive	0.019
HDL cholesterol	negative	0.069
Insulin resistance	positive	0.330

For the model $R^2 = 0.69$. These 18 diabetics (studied in chapter 4) include the 10 subjects from this chapter, and have the same entry criteria.

Table 3.23. Multiple regression analysis for the determinants of %LDL-3/ΣLDL mass ratio in the postprandial state in 18 diabetics

	Association	p value
Triglyceride AUC	positive	0.346
HDL cholesterol AUC	negative	0.154
Insulin resistance	positive	0.210

For the model $R^2 = 0.46$. These 18 diabetics (studied in chapter 4) include the 10 subjects from this chapter, and have the same entry criteria.

3.4. DISCUSSION

Hypertriglyceridaemia in diabetes, and especially in NIDDM, is likely to worsen in the postprandial state which may have adverse effects on quantity and quality of lipoprotein subfractions, not obvious from basic lipid and lipoprotein measurements. Therefore, the primary aim of this study was to examine the fasting and postprandial chylomicron, VLDL subfraction and LDL subfraction distribution and composition in NIDDM patients with hypertriglyceridaemia. In this study, modifications to the fasting VLDL subfraction profile in diabetic patients were exaggerated postprandially. The abnormal fasting LDL subfraction distribution, not apparent from total and LDL cholesterol measurements, persisted postprandially in diabetic patients.

James and Pometta (1991) showed that hypertriglyceridaemic NIDDM patients had elevated fasting levels of VLDL subfractions, reduced HDL cholesterol, a polydisperse LDL profile, and raised levels of small dense LDL (LDL-3), compared to normolipidaemic non-diabetic controls. These observations are confirmed in the present study. Elevated fasting levels of serum and VLDL triglyceride in diabetic patients could be due to increased VLDL production. Raised levels of glucose and NEFA were found in the diabetic patients, indicating insulin resistance and/or relative insulin lack. The increased NEFA flux from inadequately controlled adipose tissue lipolysis can increase hepatic triglyceride synthesis and VLDL production (Sniderman et al., 1993). It is likely that impaired clearance of VLDL also plays a role because reduced levels of HDL cholesterol present in diabetic patients is indicative of low LPL activity also due to insulin resistance and/or relative insulin lack. In the present study, β-cell function, but not insulin levels, was significantly reduced in diabetic patients, indicative of relative insulin lack. Insulin resistance was found to be substantially higher in diabetic patients compared to controls, as expected, although this difference did not reach significance. Using the HOMA method to estimate insulin resistance is less accurate than measurements obtained by the use of euglycaemic or hyperglycaemic clamp, possibly accounting for this lack of significance.

Diabetic patients had a higher proportion of the large triglyceride-rich VLDL-1 which is less readily converted to LDL (Stalenhoef et al., 1984; Packard, et al., 1984), thus providing less surface component for HDL generation. Increase in VLDL subfraction particle size was present in diabetic patients, as indicated by greater proportion of VLDL-1 at the expense of VLDL-3 and increased ratios of triglyceride/apo B and non-apo B/ apo B in each of the VLDL subfractions. Increased ratios of triglyceride/apo B and non-apo B/ apo B suggest an increase in triglyceride in the core of the lipoprotein and an increase in the

surface component of the lipoprotein respectively, both of which may be the result of an increase in triglyceride synthesis compared to apo B synthesis. Triglyceride enrichment of VLDL and increase in particle size have previously been reported in NIDDM (Taskinen et al., 1986). Large triglyceride-rich lipoproteins provide more substrate for CETP than their smaller counterparts, and consequently have greater potential for producing cholesterol ester-rich VLDL remnants. These remnants can be removed by scavenger pathways (Goldstein, 1980), resulting in foam cell formation. Although % cholesterol ester in VLDL-1 and % free cholesterol in VLDL-2 and -3 were reduced in diabetic patients, this was probably a reflection of triglyceride enrichment as confirmed by higher triglyceride/apo B ratios. Indeed, cholesterol ester/apo B ratios (data not shown) for these fractions tended to be higher in diabetic patients.

There was little difference in fasting levels of LDL cholesterol between diabetic patients and control subjects which may be due to two metabolic processes with opposing effect. Large triglyceride-rich VLDL is less readily converted to LDL, but on the other hand insulin resistance down-regulates LDL receptors on hepatocytes (Chait et al., 1979), thus reducing LDL clearance. In addition, elevated glucose levels in diabetics increases the likelihood of LDL glycation which has previously been demonstrated in moderately hypertriglyceridaemic patients with NIDDM, and has been shown to reduce LDL metabolism, thus promoting clearance via the scavenger pathway (Steinbrecher et al., 1984). Glycation may not only promote foam cell formation, but also confer increased susceptibility of LDL to oxidation (Hunt et al., 1990).

In the present study, postprandial serum and VLDL triglyceride levels and incremental increases in VLDL triglyceride were higher in diabetic patients than in controls. In addition, the postprandial VLDL triglyceride and cholesterol peaks were delayed in diabetic patients suggesting impaired clearance of triglyceride-rich lipoproteins. Incremental increases in chylomicrons and VLDL-1 were higher and peak levels delayed in diabetic patients suggesting that clearance of chylomicrons and VLDL-1 was impaired, and that this impaired clearance is reflected in postprandial serum and VLDL triglyceride levels. The VLDL-1 fraction includes chylomicron remnants as well as endogenously synthesised large VLDL. Tan and co-workers (1992) showed that chylomicron remnants were elevated in hypertriglyceridaemic subjects with NIDDM compared to normolipidaemic NIDDM patients. Even normotriglyceridaemic NIDDM patients have an excess of chylomicron remnants after an oral fat load (Chen et al., 1993). Decreased chylomicrons apo B clearance has been previously described in hypertriglyceridaemic NIDDM patients and this was concluded to be due to decrease in LPL activity (Haffner et al., 1984). Catabolism of

chylomicrons may be influenced by substrate competition with VLDL for the same saturable lipolytic pathway (Brunzel et al., 1973), the larger pool of VLDL in NIDDM reducing chylomicron metabolism. This might explain the positive association between fasting VLDL triglyceride and the magnitude of postprandial lipemia (triglyceride AUC) in diabetic patients and controls. Reduction in fasting triglyceride in NIDDM by intensive insulin therapy can improve clearance time of chylomicrons (Abrams et al., 1982). The rise in VLDL-2 levels after the test meal was higher in controls than diabetic patients, and whereas VLDL-3 levels increased in controls, they decreased in diabetic patients. It would therefore appear that in diabetic patients the production by the liver of small VLDL-3 and to a lesser extent VLDL-2 has been reduced in favour of production of larger triglyceride-rich VLDL. This would occur when the triglyceride accumulation compared to apo B synthesis is very high and in this way more triglyceride can be packaged into each VLDL particle. In controls, the accumulation of triglyceride is less allowing a greater proportion of small VLDL to be synthesised. In addition, reduced levels of small VLDL would also arise from slower degradation of the excess large VLDL and chylomicron remnants. In line with this, Karpe et al. (1993a) demonstrated a postprandial increase in large VLDL in normotriglyceridaemic and hypertriglyceridaemic patients with CHD, but a decrease in small VLDL in the hypertriglyceridaemic patients only.

Postprandial responses in insulin, glucose and NEFA were elevated and delayed in diabetic patients indicating insulin resistance and/or relative insulin lack in diabetic patients. Because NIDDM patients have relative insulin lack their insulin response to glucose is inadequate resulting in prolonged glycemia, which in turn stimulates further insulin production resulting in prolonged insulin response. Postprandial insulin responses in NIDDM have previously been reported to be delayed (Polonsky et al., 1988). The increase in insulin levels after the test meal leads to the initial fall in NEFA concentrations, due to inhibition of lipolysis in adipose tissues (Swislocki et al., 1987). In this way, hepatic VLDL production is reduced in the postprandial state to avoid competition with chylomicrons and their remnants. NEFA concentrations rise again due to the decline in insulin levels allowing lipolysis to resume. However, in diabetic patients, levels of NEFA were elevated, despite higher insulin levels; suggesting that insulin resistance and not just insulin deficiency is responsible. Insulin resistance results in impaired inhibition of lipolysis in adipose tissue, elevating plasma NEFA concentration (Frayn and Coppack, 1992) which can stimulate further VLDL synthesis, and compete for clearance with postprandial triglyceride-rich lipoproteins. Reduced suppression of lipolysis by insulin has been demonstrated in NIDDM (Fraze et al., 1985; Skowronski et al., 1991) and shown to be associated with raised levels

of triglyceride and apo B (McKeigue et al., 1993). Insulin resistance can therefore lead to elevated postprandial lipemia by stimulating VLDL production as well as by reducing chylomicron and VLDL metabolism, which might explain the positive association (albeit not significant) between insulin resistance and postprandial triglyceride response in controls and diabetics.

In both subject groups, HDL and LDL cholesterol decreased after the test meal. This may occur because the proportion of large triglyceride-rich VLDL, less readily catabolised to give rise to LDL and HDL, was higher in both subject groups after the test meal. In addition cholesterol ester transfer from HDL and LDL by CETP may have been stimulated by elevated triglyceride-rich lipoproteins as has been reported in NIDDM compared to non-diabetic subjects (Bagdade et al., 1993). Despite significant reductions in HDL cholesterol, HDL triglyceride/cholesterol ratio increased after the test meal in both subject groups (significant only in controls), indicating triglyceride enrichment of HDL. HDL may have acquired triglyceride from triglyceride-rich lipoproteins by the actions of CETP. In this way, elevated levels of large triglyceride-rich lipoproteins in diabetics may account for increased triglyceride enrichment of HDL in the fasting and postprandial state, despite lower HDL cholesterol and apo AI levels. Patsch and co-workers (1984), proposed that excessive postprandial triglyceridaemia leads to cholesterol ester depletion of HDL_2. The triglycerides that replace the cholesterol ester are rapidly hydrolysed by HL producing smaller HDL_3 particles. This might explain the lower (although not significant) HDL_2/HDL_3 cholesterol ratio in diabetic patients compared to controls.

Elevated levels of LDL-3 and %LDL-3/ΣLDL ratio persisted after the test meal in diabetic patients. Small dense LDL has been described in patients with CHD (Austin et al., 1988; Campos et al., 1992b). Contributing to their atherogenic potential, small, dense LDL have been shown to bind less well to LDL receptors compared to larger LDL (Kleinman et al., 1987a; Kleinman et al., 1987b), and are more readily oxidised than larger LDL (Tribble et al., 1992), perhaps because they carry less lipid soluble antioxidants such as vitamin E. As mentioned in Chapter one, the most probable mechanism by which small dense LDL arise is that triglyceride transfers to HDL and LDL via CETP in exchange for cholesteryl ester (Tall, 1986). LDL enriched with triglyceride becomes a substrate for lipases, which results in the formation of small, dense LDL (LDL-3) (Auwerx et al., 1988; Levy et al., 1990; Zambon et al., 1993). Kinetic studies of LDL metabolism suggest that small dense LDL are derived from large triglyceride-rich VLDL in the hypertriglyceridaemic state, whereas in the normotriglyceridaemic state, the liver produces small VLDL particles which give rise to larger, more buoyant LDL (LDL-1 and -2) (Caslake et al., 1992).

Data from the present study are consistent with, and support this hypothesis. Diabetic patients had elevated levels of small dense LDL and raised VLDL, particularly large triglyceride-rich VLDL-1. Furthermore, they had larger particle size within each VLDL subfraction, as indicated by increased non apo B protein/apo B and triglyceride/apo B ratios. Diabetic patients also had higher LDL triglyceride, and % triglyceride in LDL-2, and reduced % cholesterol in LDL-2 possibly resulting from transfer of triglyceride from elevated triglyceride-rich lipoproteins or from lipase action on triglyceride-rich lipoproteins resulting in triglyceride-rich LDL.

In the present study, in NIDDM patients, % LDL-3 /ΣLDL correlated positively with BMI, glucose, insulin resistance ($p=0.06$) and fasting and postprandial levels of triglyceride-rich lipoproteins, and correlated negatively with fasting and postprandial levels of HDL cholesterol. Correlations were stronger with fasting lipid values compared to fasting values, possibly because postprandial values are subject to more variation. The positive relationship between postprandial triglyceride-rich lipoproteins and LDL density has previously been reported in subjects with CHD (Karpe et al., 1993b). The relationship of hypertriglyceridaemia and HDL cholesterol with LDL size is well established in NIDDM (Feingold et al., 1992; Stewart et al., 1993). The negative association between HDL cholesterol and preponderance of small, dense LDL reflects the efficiency of catabolism of triglyceride-rich lipoproteins. A low HDL cholesterol would indicate a slow clearance of triglyceride-rich lipoproteins which as previously discussed, could influence LDL size distribution. The positive association between glucose and insulin resistance and preponderance of small, dense LDL confirms the findings of previous studies (Feingold et al., 1992; Selby et al., 1993; Reaven et al., 1993). Insulin resistance is associated with high serum and VLDL triglycerides in subjects with normal and impaired glucose intolerance and in NIDDM (Abbott et al., 1987; Laakso et al., 1990), which, as previously discussed, can lead to the production of small, dense LDL.

There are conflicting reports on whether small dense LDL in NIDDM arise from hypertriglyceridaemia or from factors specifically associated with diabetes. It was reported by Feingold et al., (1992) that phenotype B (predominance of small, dense LDL) was more common in normolipidaemic NIDDM patients than normolipidaemic controls, suggesting that diabetes itself plays a role in the production of small, dense LDL. In addition, two studies in non-diabetic subjects showed a strong positive relationship between insulin resistance and the appearance of small dense LDL (Reaven et al., 1993; Selby et al., 1993). The latter were associated with higher triglyceride levels, higher BMI and lower HDL cholesterol. In one study (Reaven et al., 1993), when subjected to multiple linear regression

analysis, with the inclusion of triglycerides, the relationship between insulin resistance and LDL size and was no longer significant. More recently however, it has been reported (James et al., 1994) that LDL-2/LDL-3 mass ratio was negatively associated with fasting levels of glucose and HbA1c both remaining significantly correlated with LDL-2/LDL-3 mass ratio when corrected for triglycerides. Also, multiple linear regression analysis, including triglycerides and HDL cholesterol, showed that LDL-2/LDL-3 mass ratio correlated with HbA1c. In the present study, multivariate analysis confirmed the findings of univariate analysis, by showing that the strength of the correlations were weaker with insulin resistance compared to serum triglyceride and HDL cholesterol, suggesting that hypertriglyceridaemia has greater influence than NIDDM on the prevalence of small, dense LDL.

In summary, this study has demonstrated abnormalities in the fasting and postprandial composition and distribution of lipoprotein subfractions in hypertriglyceridaemic patients with NIDDM. Elevated fasting levels of large triglyceride-rich lipoproteins partly due to insulin resistance and/or relative insulin lack in NIDDM patients were exacerbated postprandially. Triglyceride enrichment of LDL and HDL, raised levels of small, dense LDL, and lower HDL cholesterol concentrations persisted postprandially. These abnormalities contribute significantly to the increased risk of atherogenesis in hypertriglyceridaemic patients with NIDDM.

CHAPTER 4

EFFECTS OF BEZAFIBRATE ON FASTING AND POSTPRANDIAL LIPAEMIA IN NIDDM

4.1. INTRODUCTION

The fibric acid group of hypolipidaemic drugs have been associated with significant reductions in coronary events in the Helsinki Heart study, especially in patients with combined hyperlipidaemia (Manninen et al., 1987). Fibric acid derivatives have been recommended for the treatment of diabetic hyperlipidaemia (Durrington and Winocour 1989; Winocour and Laker 1990). These agents have the ability to lower triglyceride levels and increase HDL by increasing LPL activity, which would be clearly beneficial for treatment of dyslipidaemia in NIDDM.

In chapter three, patients with NIDDM were shown to have many quantitative and qualitative lipoprotein abnormalities including elevated fasting levels of triglyceride-rich lipoproteins which were exacerbated postprandially, as well as increased levels of small dense LDL and reduced levels of HDL cholesterol in both the fasting and postprandial state. Diabetic patients had an increased proportion of large VLDL, and particle size was greater within each VLDL subfraction compared to controls. Furthermore, HDL and LDL was triglyceride-enriched in diabetic patients. These findings clearly emphasise the potential importance of lowering triglyceride levels in patients with NIDDM. Bezafibrate has been used widely in the treatment of hyperlipidaemia including patients with NIDDM (Seviour et al., 1988; Rovellini et al., 1992; Niort et al., 1992). However, these study and earlier studies of bezafibrate in NIDDM have only investigated basic fasting lipid and lipoprotein measurements.

The aim of this double-blind placebo-controlled study was to investigate the effects of bezafibrate on the quantity and quality of fasting and postprandial lipids, lipoproteins, and lipoprotein subfractions in patients with NIDDM.

4.2. METHODS

4.2.1. Study Population

18 patients with moderate dyslipidaemia but satisfactory glycaemic control (Table 4.1 and 4.2), and treated with diet and glibenclamide (5-15 mg) daily were studied. These 18 patients include the 10 patients described in chapter 3. All patients received (single blind) placebo run-in for 8 weeks before the first visit, and then received either (double-blind) placebo or bezafibrate (400mg daily of Bezalip Mono Slow Release) for 8 weeks before the second visit. Ethical approval was obtained from the institutional review committee (Bath District Research Ethics Committee).

Table 4.1. Inclusion and exclusion criteria for patients.

	INCLUSION	EXCLUSION
NIDDM	Fructosamine < 340 μmol/L Cholesterol: 5.2 -8.5 mM Triglyceride: 1.8-4.5 mM	Metformin, Cyclical HRT, Renal and Hepatic diseases, Other significant disease

HRT: Hormone Replacement Therapy.

Table 4.2. Demographic data for patients.

	PLACEBO GROUP	BEZAFIBRATE GROUP
NUMBER	9	9
AGE ± SEM (yrs)	59.3± 2.92	54.2±2.23
GENDER	8 male, 1 female	7 male, 2 female
BMI ± SEM ($kg.m^{-2}$)	27.3± 0.59	28.1±1.50

4.2.2. Protocol

1. Fulfil entry criteria.
2. Written informed consent.
3. A) First visit: 8 weeks run-in on placebo (single-blind) with stable glycaemic control and lipid parameters.

   Or:-

   B) Second visit: 8 weeks on placebo or bezafibrate (double-blind) with stable glycaemic control and lipid parameters.
4. 0800 hours: Fasting blood sample.
5. 0815 hours: Standard low fat breakfast followed by normal daily activity.

6. 1200 hours: Standard low fat lunch provided.
7. 1800 hours: Admitted for overnight study.
8. 1845 and 1855 hours: Blood samples taken to check that lipid levels were similar to fasting levels.
9. 1900 hours: Test meal: mixed meal, high fat (50g fat, 1000 calories).
10. Blood samples were taken at hourly intervals for 12 hours after the test meal.

4.2.3. Measurements

Cholesterol and triglyceride in serum and serum lipoproteins (VLDL, HDL, HDL_2 and HDL_3), serum apolipoproteins (AI and B), glucose, insulin, NEFA were measured at every time interval (data supplied by C.Stirling and co-workers). Chylomicrons, LDL subfractions (LDL-1, LDL-2 and LDL-3) and VLDL subfractions (VLDL-1, VLDL-2 and VLDL-3) were isolated from plasma sampled at fasting, 3 and 8 hours after the test meal. Measurements for each subfraction included total cholesterol, triglyceride, phospholipid, free cholesterol, esterified cholesterol and total protein. The mass of each fraction was calculated as the sum of the latter five components. Composition of these fractions was expressed as a percentage of total mass. Fasting apo B concentrations were determined in VLDL-1, -2 and -3 and LDL-1 fractions only (see Methods 2.3.7). Cholesterol and triglyceride values for VLDL and LDL were calculated as the sum of these lipid components in VLDL and LDL subfractions respectively. Areas under curves (AUCs) were calculated using the trapezoid rule. Incremental areas under curves (IAUCs) were calculated by subtracting the baseline value, extrapolated over time, from the AUC value. Insulin resistance and β-cell function % were assessed using the HOMA method (see Methods section).

4.2.4. Statistics

Comparisons between treatment groups were made using an unpaired t-test and comparisons within treatment groups before and after therapy were made using a paired t-test. Measurements for triglyceride, glucose, insulin, insulin resistance, and β-cell function were log-transformed prior to analysis. Non-parametric data, which could not be log-transformed (IAUC for triglyceride, glucose, and insulin) were compared by Mann-U-Whitney test (unpaired), Wilcoxon test (paired). Data are presented as mean ± SEM. The following convention is used to indicate levels of statistical significance:

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared with start.

d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared with bezafibrate group.

4.3. RESULTS

4.3.1. Fasting lipids, lipoproteins and metabolites in bezafibrate- and placebo-treated groups

Fasting lipids, lipoproteins and metabolites results are shown in Table 3. There were no significant differences in any of these parameters before treatment between the bezafibrate-treated group and the placebo group. Following bezafibrate therapy, there were significant reductions in serum apo B (-21%) and VLDL cholesterol (-51%) and triglyceride (-45%), and a 27% reduction in serum triglyceride ($p=0.06$). There were no significant differences in cholesterol and triglyceride in LDL, HDL, HDL_2 and HDL_3, or apo AI after bezafibrate treatment. There were, however, trends to improve serum (4%) and LDL cholesterol (10%) . Bezafibrate had no significant effects on insulin, glucose or NEFA levels, although insulin levels approximately doubled in both patient groups, reaching statistical significance in the placebo group.

Table 4.3. Lipids, lipoproteins, apolipoproteins and metabolites in the bezafibrate and placebo groups.

	Bezafibrate		Placebo	
	Start	end	Start	End
Cholesterol (mM)	6.63±0.44	6.38±0.31	7.14±0.39	7.23±0.45
Triglyceride (mM)	3.31±0.46	2.40±0.39	2.80±0.35	2.66±0.45
VLDL cholesterol (mM)	1.19±0.25	0.58±0.11 a	0.90±0.12	0.80±0.12
VLDL triglyceride (mM)	2.11±0.38	1.17±0.25 a	1.57±0.26	1.67±0.22
LDL cholesterol (mM)	3.13±0.39	2.82±0.18	3.80±0.36	3.70±0.42
LDL triglyceride (mM)	0.31±0.03	0.30±0.02	0.28±0.04	0.31±0.02
HDL cholesterol (mM)	0.97±0.07	0.97±0.10	1.00±0.08	1.08±0.09
HDL_2 cholesterol (mM)	0.35±0.08	0.27±0.03	0.32±0.04	0.33±0.04
HDL_3 cholesterol (mM)	0.61±0.04	0.71±0.09	0.69±0.08	0.75±0.07
HDL triglyceride (mM)	0.29±0.03	0.27±0.03	0.21±0.08	0.20±0.02
HDL_2 triglyceride (mM)	0.07±0.02	0.07±0.02	0.05±0.01	0.05±0.01
HDL_3 triglyceride (mM)	0.22±0.02	0.20±0.03	0.18±0.02	0.21±0.04
Apo B (mg/dl)	121±8.7	96.0±9.90 a	118±7.4	118±9.06
Apo AI (mg/dl)	144±4.7	154±8.9	151±6.6	158±7.03
Glucose (mM)	8.44±0.84	7.88±0.69	10.0±0.64	9.61±0.84
Insulin (mU/l)	14.1±4.31	28.6±6.98	10.5±1.80	20.2±2.81 b
NEFA (mM)	0.73±0.08	0.68±0.09	0.75±0.05	0.64±0.11

All values are mean ± SEM. a: $p<0.05$; b: $p<0.01$ compared with start.

4.3.2. Fasting chylomicrons, VLDL subfractions and LDL subfractions in bezafibrate and placebo groups

Concentrations (mass) of chylomicrons, VLDL-1, -2 and -3, LDL-1, -2 and -3 are shown in Fig. 4.1a and 4.1b, and lipid and protein components of these lipoproteins in Tables 4.4 and 4.5. Bezafibrate treatment resulted in significant reductions in VLDL-1 (-51%) and VLDL-2 mass (-49%) (Fig. 1a) which were reflected in reductions of total protein and all lipid components of these subfractions (Table 4.4). In addition, there was a significant reduction in VLDL-3 triglyceride (-40%) following bezafibrate therapy (Table 4.4). There was a significant decrease in LDL-1 esterified cholesterol and increase in LDL-2 free cholesterol following bezafibrate therapy, and it is interesting to note that the LDL profile was less polydisperse, which could not be said of the placebo group (Table 4.5). The placebo group had significantly higher levels of LDL-3 compared to the bezafibrate-treated group before and after treatment, which was reflected by higher total LDL cholesterol in the placebo group.

Composition (expressed as a percentage of total mass) for chylomicrons, VLDL

subfractions and LDL subfractions are shown in Tables 4.6 and 4.7. Percent protein in chylomicrons was significantly increased following bezafibrate therapy, which was reflected in a reduction in % triglyceride (Table 4.6). Percent esterified cholesterol in VLDL-1, -2 and -3 fell significantly in the bezafibrate-treated group which was reflected in a significant increase in triglyceride in VLDL-1 and -2 (Table 4.6). There was little in the way of compositional differences in LDL subfractions (Table 4.7).

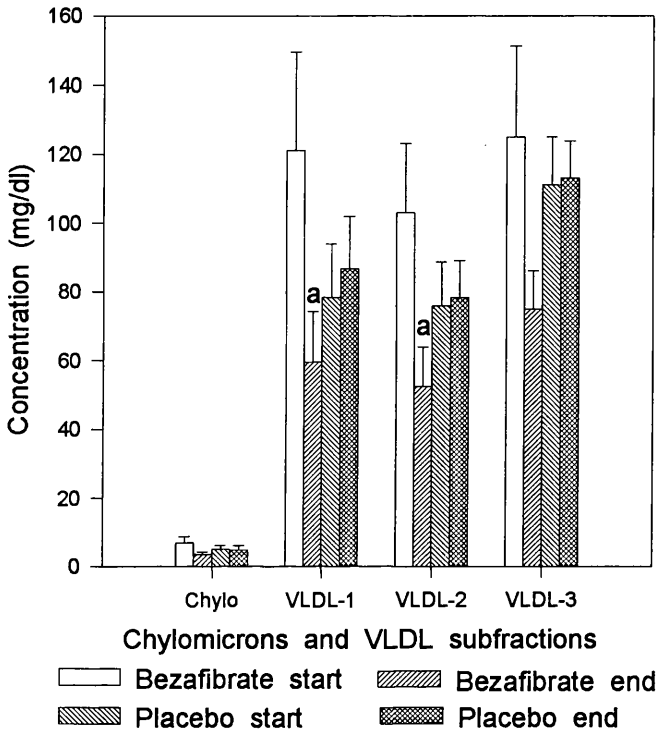


Fig. 4.1a Concentrations of chylomicrons (Chylo), and VLDL subfractions in bezafibrate and placebo groups. All values are mean ± SEM. a: $p<0.05$ compared with start.

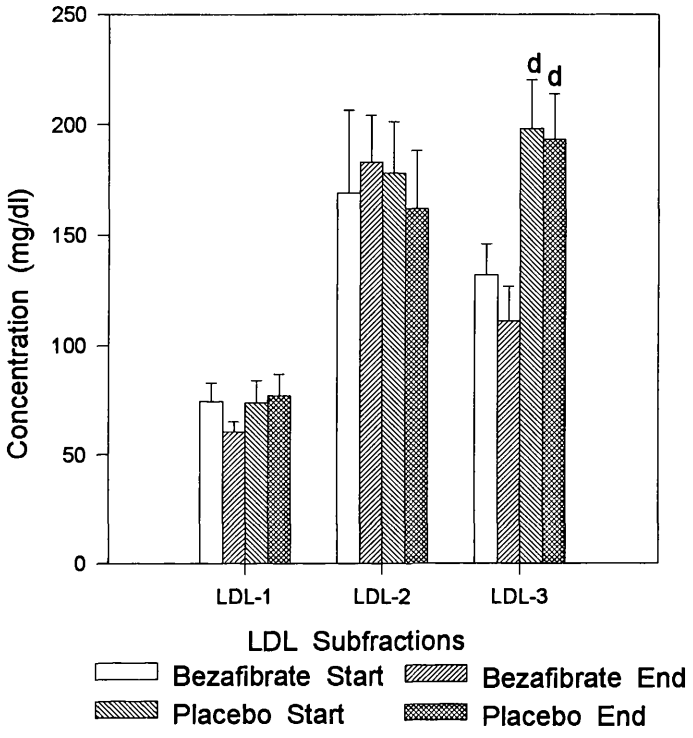


Fig. 4.1b: Concentrations of LDL subfractions in bezafibrate and placebo groups.

All values are mean ± SEM. d: $p<0.05$ compared with bezafibrate group.

Table 4.4. Fasting concentrations of lipid and protein components of chylomicrons and VLDL subfractions in bezafibrate and placebo groups.

	Chylomicrons				VLDL-1				VLDL-2				VLDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	0.59± 0.23	0.19± 0.05	0.37± 0.09	0.37± 0.08	10.1± 2.58	4.05± 0.99 a	5.53± 1.05	6.79± 1.55	12.3± 2.73	5.15± 1.15 a	8.16± 1.56	8.06± 1.27	23.6± 6.05	13.3± 2.40	20.9± 2.60	20.1± 2.15
TG	5.13± 1.33	2.20± 0.71	3.59± 0.85	3.22± 1.17	80.8± 18.8	41.9± 10.4 a	51.9± 10.8	57.4± 9.53	47.1± 10.7	32.2± 7.09 a	42.9± 7.31	44.8± 5.95	49.3± 8.70	29.4± 5.03 a	44.4± 6.44	45.4± 5.68
PL	0.45± 0.15	0.24± 0.07	0.41± 0.11	0.42± 0.10	15.0± 4.09	6.79± 1.80 a	10.7± 2.48	11.4± 2.58	15.6± 3.53	6.84± 1.74 a	12.2± 2.40	12.1± 2.14	21.6± 5.19	13.3± 2.30	20.0± 2.68	20.9± 2.27 d
FC	0.17± 0.07	0.06± 0.03	0.08± 0.04	0.09± 0.03	5.13± 1.38	2.37± 0.65 a	3.41± 0.64	4.14± 1.01	4.85± 0.97	2.61± 0.63 b	4.16± 0.77	4.71± 0.92	8.62± 2.09	5.81± 1.06	8.71± 1.10	9.20± 1.08 d
EC	0.71± 0.28	0.22± 0.08	0.49± 0.14	0.47± 0.13	8.43± 2.14	2.83± 0.60 a	3.57± 0.75 d	4.46± 0.93	12.6± 3.39	4.27± 0.91 a	6.73± 1.39	5.63± 0.91	25.2± 6.75	12.5± 2.32	20.5± 2.83	18.2± 2.27
Prot	0.37± 0.10	0.64± 0.26	0.48± 0.17	0.60± 0.17	11.32± 2.58	5.48± 1.32 a	8.71± 1.42	9.25± 1.64	12.7± 2.50	6.56± 1.32 a	9.83± 1.50	11.1± 1.77	20.4± 4.65	13.8± 1.50	17.2± 2.46	19.1± 1.09 d
Apo B					4.45± 1.20	2.50± 0.71	3.84± 0.66	4.26± 0.74	5.47± 1.17	3.48± 0.78 a	4.19± 0.72	5.68± 1.14	13.5± 3.42	10.3± 1.22	10.4± 1.94	12.4± 0.68
Mass	6.82± 1.74	3.37± 0.84	5.05± 1.04	4.80± 1.33	121± 28.5	59.4± 14.7 a	78.3± 15.6	86.7± 15.2	103± 20.1	52.5± 11.5 a	75.8± 13.0	78.3± 10.7	125± 26.3	74.9± 11.2	111± 13.9	113± 10.9

All values are mean ± SEM (mg/dl).

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$ compared with start.

d: $p<0.05$ compared with bezafibrate group.

Table 4.5. Fasting concentrations of lipid and protein components of LDL subfractions in bezafibrate and placebo groups.

	LDL-1				LDL-2				LDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	23.0± 2.92	17.7± 1.60	22.8± 3.56	24.6± 4.50	55.7± 13.5	56.8± 6.89	60.2± 8.27	55.3± 10.2	42.5± 4.93	34.9± 5.17	64.1± 7.09 d	62.7± 7.02 e
TG	11.0± 1.24	9.49± 0.72	10.1± 1.12	10.8± 1.21	10.5± 1.51	12.1± 1.27	8.74± 1.26	9.63± 0.86	6.27± 0.68	5.03± 0.73	6.06± 1.20	6.91± 0.56
PL	14.9± 1.60	12.5± 1.27	15.6± 2.87	14.5± 1.88	35.2± 8.91	39.0± 4.97	25.7± 4.70	3.9± 5.93	23.7± 2.58	21.7± 3.06	36.8± 4.36 d	36.3± 4.29 d
FC	6.74± 0.85	6.04± 0.53	7.24± 0.84	7.07± 0.85	15.4± 4.32	18.5± 2.45 a	15.8± 1.77	16.4± 3.03	9.34± 1.20	12.5± 2.39	15.2± 1.43 e	15.4± 1.60
EC	27.3± 3.60	19.5± 1.98 a	26.2± 4.69	29.4± 6.77	67.8± 15.7	64.3± 7.90	74.7± 11.0	65.3± 12.4	55.8± 6.38	37.6± 7.54	82.2± 9.65 d	79.5± 9.22 e
Prot	14.4± 1.93	12.7± 0.88	14.7± 2.00	15.1± 1.46	40.6± 8.08	49.6± 6.32	43.1± 5.64	40.4± 6.81	37.1± 4.08	34.0± 4.60	58.0± 6.76 d	55.3± 5.84 d
Apo B	13.1± 1.82	11.3± 0.79	12.8± 1.80	12.6± 1.41								
Mass	74.3± 8.48	60.2± 4.63	73.8± 10.2	76.8± 9.93	169± 37.5	183± 21.1	178± 23.2	162± 26.0	132± 13.9	111± 15.8	198± 22.2 d	193± 20.7 d

All values are mean ± SEM (mg/dl).

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$ compared with start.

d: $p<0.05$; e: $p<0.01$ compared with bezafibrate group.

Table 4.6. Fasting composition of chylomicrons and VLDL subfractions in bezafibrate and placebo groups.

	Chylomicrons				VLDL-1				VLDL-2				VLDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	10.6± 2.35	8.05± 2.89	6.99± 0.71	10.8± 3.47	8.28± 0.47	6.92± 0.21 a	8.57± 1.43	6.88± 0.82	11.7± 0.44	9.45± 0.40 b	9.96± 0.83	9.83± 0.74	17.9± 0.81	16.8± 1.42	19.0± 0.89	17.6± 0.89
TG	73.3± 2.63	55.8± 7.81	68.0± 6.07	53.1± 8.09	68.2± 1.41	71.1± 1.15 b	61.7± 4.21	68.3± 2.16	57.0± 1.81	61.4± 1.37 b	56.6± 0.98	58.3± 2.20	40.8± 1.50	38.2± 2.30	39.3± 1.72	39.6± 1.84
PL	5.55± 1.05	9.79± 2.72	9.71± 3.29	13.0± 3.27	10.8± 1.35	10.1± 0.95	12.0± 1.36	12.3± 1.05	14.1± 1.33	11.5± 1.29	15.5± 0.79	15.1± 0.97	17.2± 1.20	16.9± 1.35	18.0± 0.52	18.4± 0.90
FC	2.95± 0.91	3.38± 1.94	1.45± 0.58	1.63± 0.68	3.82± 0.29	3.67± 0.43	5.61± 1.20	4.07± 0.62	4.64± 0.34	4.48± 0.56	4.97± 0.56	5.77± 0.54	6.78± 0.39	7.33± 0.74	7.81± 0.22	8.04± 0.41
EC	12.8± 3.77	7.85± 2.33	9.31± 1.14	15.4± 6.10	7.48± 0.76	5.47± 0.88 a	4.97± 0.64 d	4.72± 0.37	11.9± 1.17	8.35± 0.65 a	8.38± 0.87 d	6.83± 0.89	18.7± 1.31	15.8± 1.27 a	18.9± 1.41	16.0± 1.30 a
Prot	5.38± 1.08	23.2± 6.48 a	11.8± 4.21	16.9± 4.87	9.67± 0.73	9.75± 0.67	15.7± 3.88	10.6± 0.42	12.4± 0.92	14.2± 1.25	14.5± 1.43	14.0± 0.83	16.5± 1.46	21.8± 4.27	16.1± 1.36	18.0± 1.93

All values are mean % of total mass ± SEM.

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$ compared with start.

d: $p<0.05$ compared with bezafibrate group.

Table 4.7. Fasting composition of LDL subfractions in bezafibrate and placebo groups.

	LDL-1				LDL-2				LDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	30.5± 0.89	29.1± 0.82	30.6± 1.20	30.6± 1.64	31.5± 0.86	31.1± 0.98	33.5± 0.69	33.2± 0.89	32.0± 0.61	31.3± 0.71	32.7± 0.71	32.3± 0.55
TG	15.2± 1.28	16.2± 1.20	14.9± 1.81	15.1± 1.78	7.34± 0.81	6.69± 0.43	5.29± 0.74	6.63± 0.73	4.86± 0.34	4.81± 0.66	5.28± 0.62	3.78± 0.33
PL	20.5± 1.08	20.4± 0.72	20.6± 1.01	19.0± 1.04	19.5± 0.99	21.1± 0.92	20.0± 0.59	20.6± 0.57	18.0± 0.70	19.7± 0.71	19.5± 0.54	18.7± 0.37
FC	9.08± 0.40	10.0± 0.44	10.0± 0.36	9.42± 0.59	8.47± 0.48	10.0± 0.35 a	9.08± 0.32	9.94± 0.51	6.97± 0.36	12.0± 2.62	8.08± 0.45	8.02± 0.28
EC	36.0± 1.55	32.0± 1.62	34.6± 2.23	35.7± 3.42	38.8± 1.18	35.4± 1.81	41.1± 1.48	39.2± 1.39	42.0± 0.88	32.4± 3.97	41.4± 1.06	40.8± 1.12
Prot	19.3± 1.03	21.3± 0.97	20.0± 0.70	20.8± 1.63	26.0± 1.80	26.7± 1.10	24.5± 0.95	23.6± 0.96 d	28.1± 1.31	31.1± 1.03	25.7± 1.46	28.8± 0.81

All values are mean % of total mass ± SEM.

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$ compared with start.

d: $p<0.05$ compared with bezafibrate group.

A qualitative comparison of lipoproteins and lipoprotein subfractions and metabolites is shown in Table 4.8. Percentage LDL-3 in total LDL (%LDL-3/∑LDL), indicative of the proportion of small dense LDL, fell significantly after bezafibrate therapy and was significantly lower than the placebo group. Percentage VLDL-3 in total VLDL (%VLDL-3/ ∑VLDL) significantly increased in the bezafibrate-treated group which was reflected in non-significant reductions in the larger triglyceride-rich VLDL subfractions. There were no significant differences in triglyceride enrichment of HDL, in ratios of HDL_2/HDL_3 and LDL/HDL cholesterol, or in VLDL subfraction particle size (indicated by triglyceride/apo B and non-apo B/apo B ratios) following bezafibrate treatment. Insulin resistance and β-cell function were not significantly altered after bezafibrate therapy, however, it is interesting to note that these parameters increased in both treatment groups, reaching significance for insulin resistance in the placebo group.

Table 4.8. Fasting qualitative parameters in bezafibrate and placebo groups.

	Bezafibrate		Placebo	
	Start	end	Start	end
%VLDL-1/ ∑VLDL	32.0±4.23	27.7±3.18	26.1±3.44	29.0±2.50
%VLDL-2/ ∑VLDL	28.7±1.07	25.9±1.66	27.3±1.89	27.9±1.18
%VLDL-3/ ∑VLDL	39.4±4.70	46.3±4.23 a	46.6±4.86	43.1±2.96
%LDL-3/∑LDL	37.7±4.36	31.0±4.31 b	44.4±3.21	45.6±3.36 d
LDL chol/ HDL chol	3.23±0.34	3.05±0.22	3.89±0.36	3.40±0.26 b
HDL_2/ HDL_3 chol	0.63±0.19	0.44±0.09	0.53±0.11	0.48±0.08
HDL trig/chol	0.32±0.05	0.34±0.08	0.23±0.03	0.21±0.01
HDL_2 trig/chol	0.25±0.06	0.25±0.07	0.16±0.03	0.17±0.03
HDL_3 trig/chol	0.38±0.03	0.38±0.09	0.28±0.04	0.29±0.05
Insulin resistance	5.11±1.60	10.9±2.98	4.64±0.84	8.95±1.69 a
β-cell function %	105±45.7	137±29.3	35.2±7.49	73.4±10.7
VLDL-1 trig/apo B	22.2±4.68	23.0±6.16	14.9±5.00	14.6±1.52
VLDL-2 trig/apo B	11.8±1.42	9.62±0.77	11.2±1.40	9.88±1.64
VLDL-3 trig/apo B	4.15±0.51	2.98±0.40	5.18±1.10	3.70±0.42
VLDL-1 Non-apoB/apo B	1.91±0.49	1.83±0.58	1.49±0.35	1.25±0.18
VLDL-2 Non-apoB/apo B	1.42±0.16	1.16±0.19	1.86±0.49	1.23±0.27
VLDL-3 Non-apoB/apo B	0.56±0.07	0.38±0.05	0.84±0.15	0.54±0.05 d

All values are mean ± SEM.
Chol: Cholesterol; Trig: Triglyceride; Non-apo B: Non-apo B protein.
a: $p<0.05$; b: $p<0.01$ compared with start; d: $p<0.05$ compared with bezafibrate group.

4.3.3. Postprandial lipids, lipoproteins and metabolites in bezafibrate and placebo groups

Postprandial responses for lipids, lipoproteins and metabolites are shown in Figs. 4.2-4.9 and the calculated areas under the postprandial curves (AUC) together with the incremental areas under the curves (IAUC) are shown below each Figure in Tables 4.9-4.16 respectively.

While there were no differences in the time to reach peak triglyceride levels (Fig. 2) following bezafibrate therapy, AUC for serum triglyceride fell significantly (-35%) (Table 4.9), with an accompanying (non-significant) reduction in AUC for cholesterol (-12%) (Table 4.9).

AUCs for VLDL cholesterol and triglyceride and serum apo B fell significantly by 38%, 63% and 27% respectively in the bezafibrate-treated group and were significantly lower than the placebo group (Table 4.10 and 4.14). IAUC for VLDL triglyceride significantly decreased (-74%) after bezafibrate therapy (Table 4.10) reflected by a reduction in peak VLDL triglyceride time from 6-9 hours to 3-8 hours (Fig. 4.3). There were no significant postprandial differences in HDL, HDL_2 and HDL_3 cholesterol and triglyceride or apo AI in response to bezafibrate therapy (Fig. 4.4-4.7, Tables 4.11-4.14).

Following bezafibrate therapy, AUC for NEFA was significantly reduced (20%) (Table 4.15). There was no significant difference in AUC for glucose in response to bezafibrate therapy (Table 4.15), and although IAUC for glucose was significantly raised (Table 4.15), the difference was extremely small (Fig. 4.8). AUC and IAUC for insulin were not significantly altered after bezafibrate treatment although these parameters significantly increased in the placebo-treated group (Table 4.16).

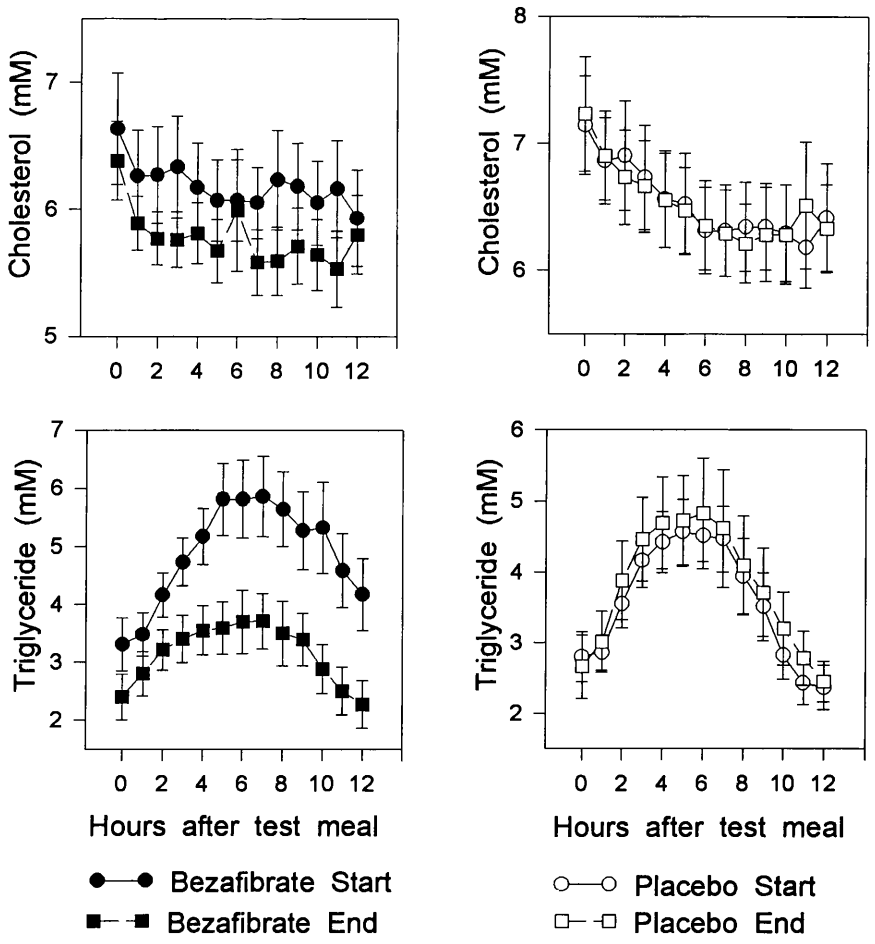


Fig. 4.2. Fasting and postprandial concentrations of cholesterol and triglyceride in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
Cholesterol (mM.hrs) AUC	71.5±4.32	63.1±2.90	74.9±4.01	71.1±3.97
Cholesterol (mM.hrs) IAUC	-8.12±2.51	-13.4±1.49	-10.8±1.64	-15.6±1.99 a
Triglyceride (mM.hrs) AUC	59.6±5.83	38.6±4.94 a	43.8±3.97	46.5±6.63
Triglyceride (mM.hrs) IAUC	19.8±3.77	9.77±1.55	10.2±2.13 d	14.6±2.00

Table 4.9. AUC and IAUC for cholesterol and triglyceride in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 12 hours. AUCs were calculated as areas above zero concentration, while IAUCs were calculated as net areas above or below (+ or -) the concentration at basal time for this Table and subsequent Tables.

Beza: bezafibrate

a: $p<0.05$ compared with start.

d: $p<0.05$ compared with bezafibrate group.

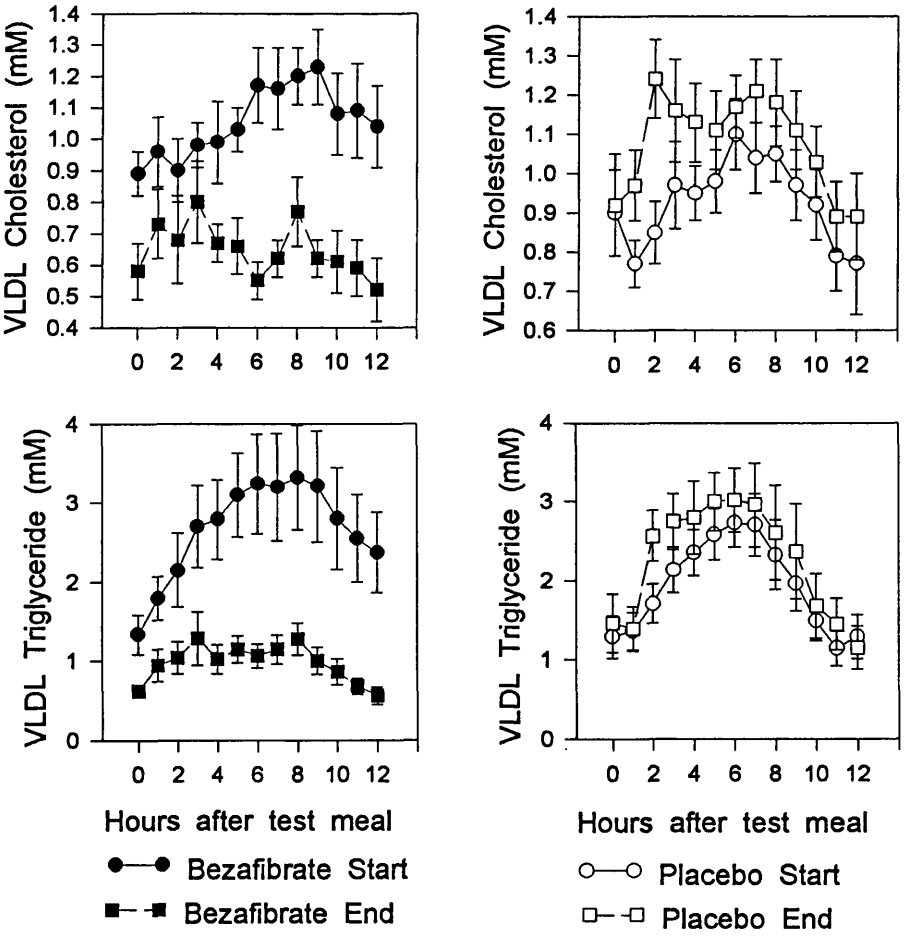


Fig. 4.3. Fasting and postprandial concentrations of VLDL cholesterol and triglyceride in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
VLDL chol (mM.hrs) AUC	12.7±1.14	7.86±0.73 c	11.2±0.67	13.2±0.95 f
VLDL chol (mM.hrs) IAUC	2.11±0.59	0.92±0.60	0.40±0.86	2.17±1.38
VLDL trig (mM.hrs) AUC	32.7±5.65	12.0±1.42 a	23.8±2.79	27.9±4.47 e
VLDL trig (mM.hrs) IAUC	16.8±3.83	4.52±1.49 a	8.36±0.98	10.4±3.63 d

Table 4.10. AUC and IAUC for VLDL cholesterol and triglyceride in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 12 hours.
Beza: bezafibrate
a: $p<0.05$; c: $p<0.001$ compared with start.
d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared with bezafibrate group.

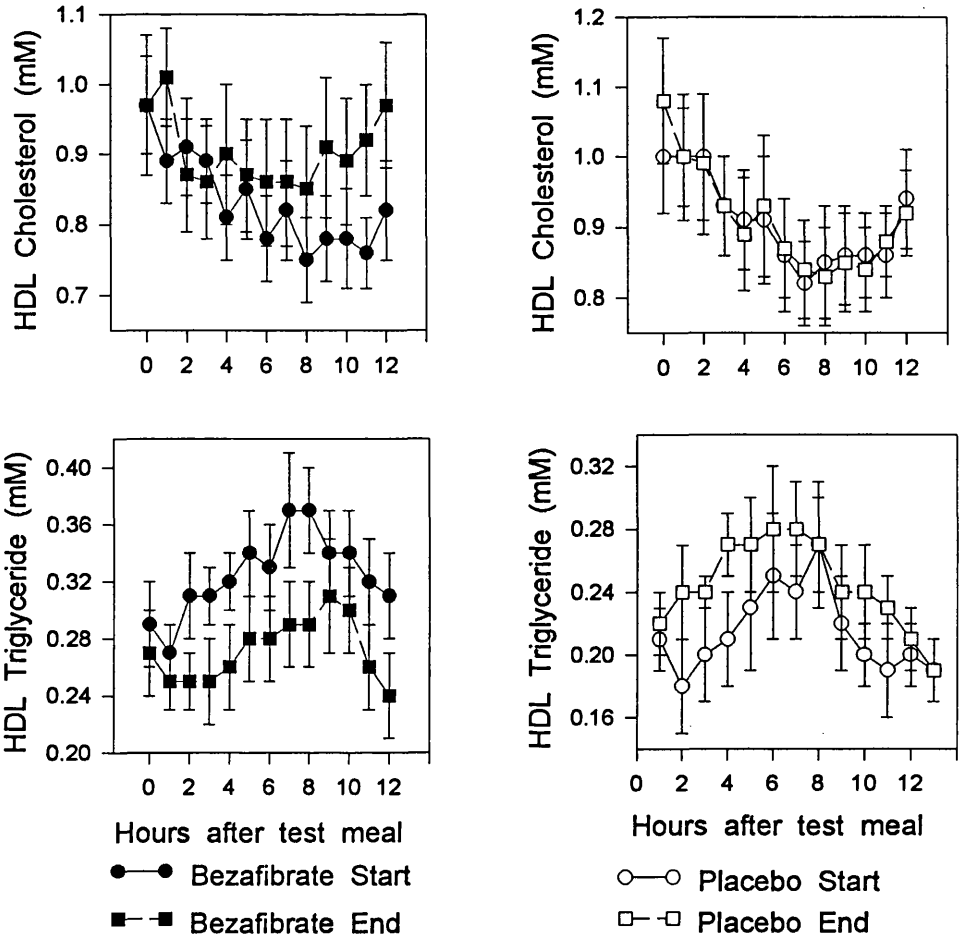


Fig. 4.4. Fasting and postprandial concentrations of HDL cholesterol and triglyceride in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
HDL chol (mM.hrs) AUC	9.91±0.71	10.8±1.00	10.8±0.85	10.8±0.85
HDL chol (mM.hrs) IAUC	-1.67±0.59	-0.92±0.55	-1.17±0.24	-2.14±0.37
HDL trig (mM.hrs) AUC	3.92±0.28	3.28±0.30	2.59±0.31 d	2.97±0.22
HDL trig (mM.hrs) IAUC	0.41±0.28	-0.02±0.31	0.05±0.21	0.29±0.31

Table 4.11. AUC and IAUC for HDL cholesterol and triglyceride in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 12 hours.
Beza: bezafibrate
d: $p<0.05$ compared with bezafibrate group.

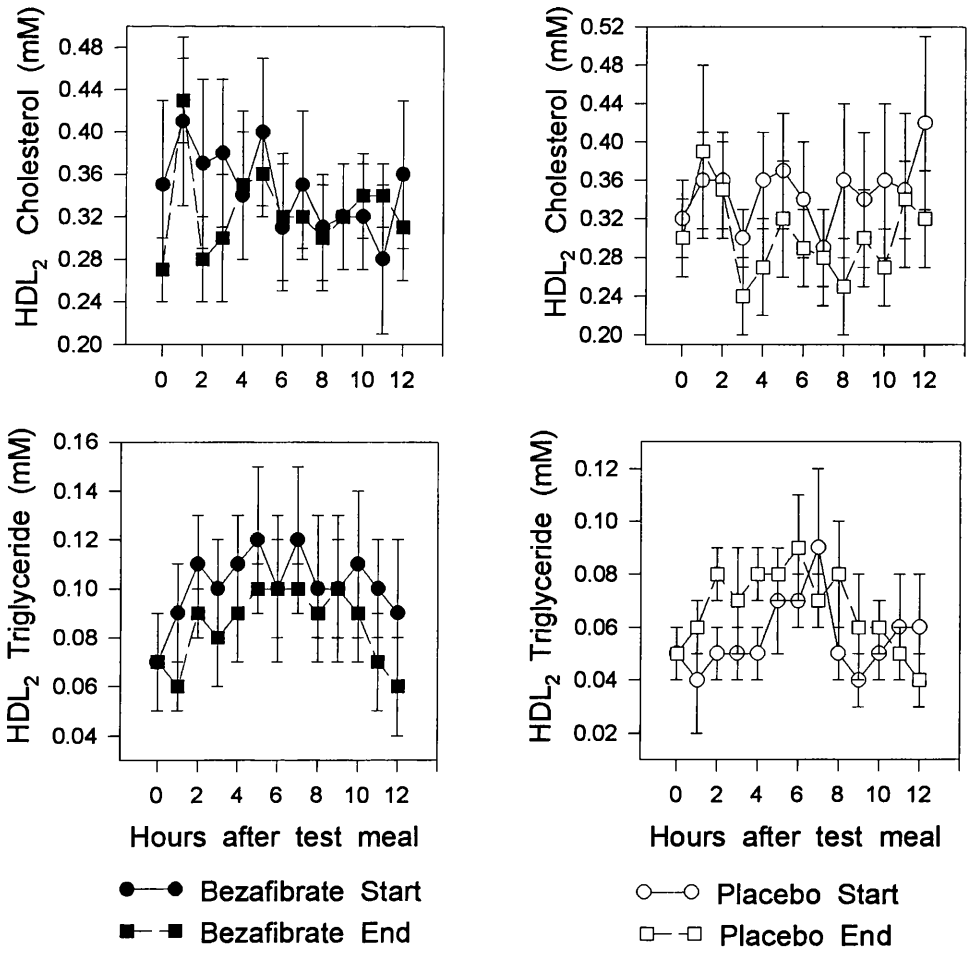


Fig. 4.5. Fasting and postprandial concentrations of HDL_2 cholesterol and triglyceride in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
HDL_2 chol (mM.hrs) AUC	4.14±0.72	3.95±0.45	4.17±0.52	3.64±0.47
HDL_2 chol (mM.hrs) IAUC	-0.10±0.55	0.75±0.44	0.38±0.40	-0.36±0.30
HDL_2 trig (mM.hrs) AUC	1.70±0.35	1.38±0.19	0.91±0.14	1.12±0.16
HDL_2 trig (mM.hrs) IAUC	0.82±0.28	0.54±0.10	0.37±0.16	0.48±0.17

Table 4.12. AUC for IAUC for HDL_2 cholesterol and triglyceride in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 12 hours.
Beza: bezafibrate

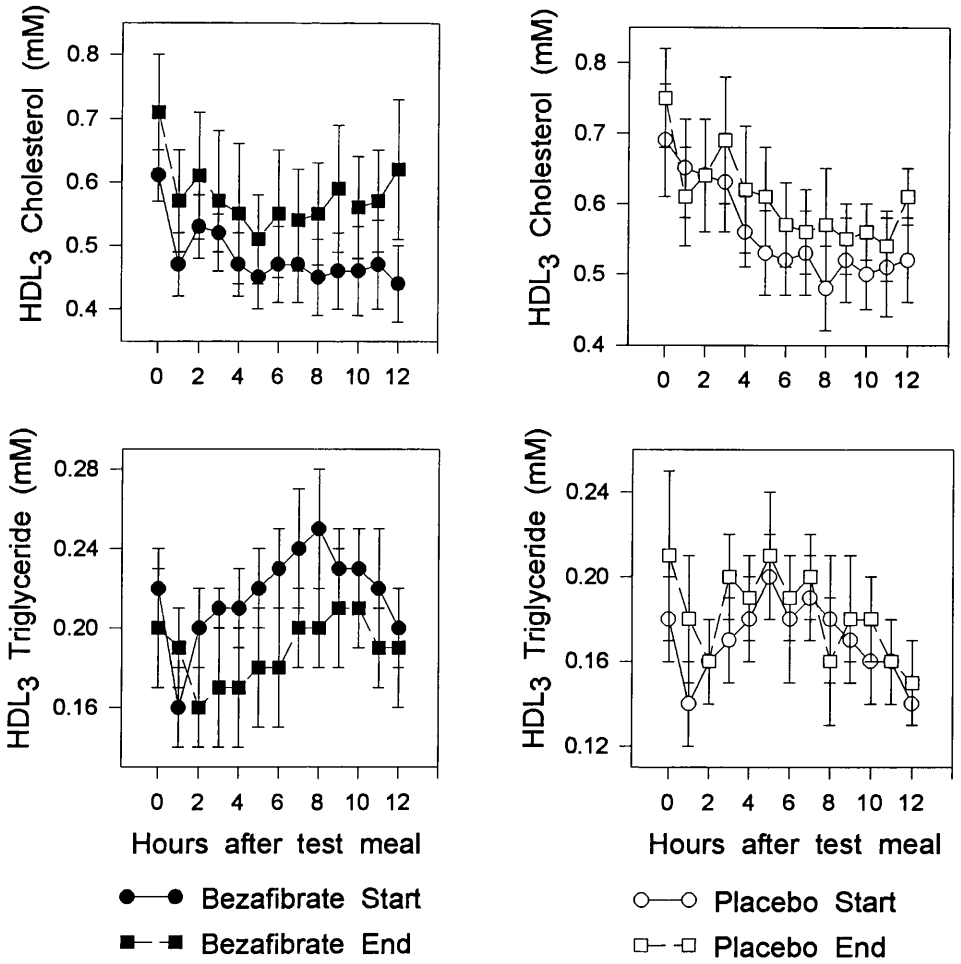


Fig. 4.6. Fasting and postprandial concentrations of HDL_3 cholesterol and triglyceride in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
HDL_3 chol (mM.hrs) AUC	5.75±0.58	6.82±1.02	6.67±0.60	7.21±0.67
HDL_3 chol (mM.hrs) IAUC	-1.59±0.36	-1.68±0.25	-1.55±0.49	-1.78±0.33
HDL_3 trig (mM.hrs) AUC	2.64±0.21	2.24±0.24	2.05±0.22	2.16±0.19
HDL_3 trig (mM.hrs) IAUC	-0.01±0.16	-0.21±0.29	-0.06±0.14	-0.37±0.55

Table 4.13. AUC and IAUC for HDL_3 cholesterol and triglyceride in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 12 hours.
Beza: bezafibrate

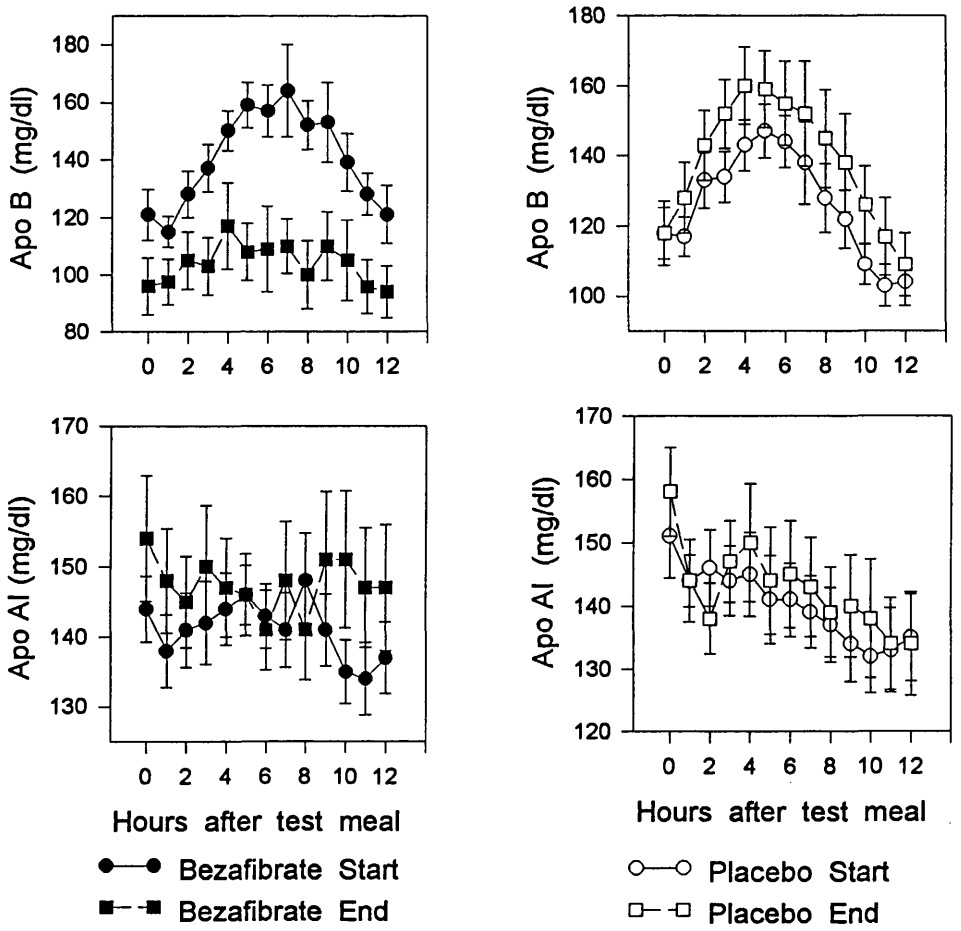


Fig. 4.7. Fasting and postprandial concentrations of Apo B and Apo AI in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
Apo B (mg/dl.hrs) AUC	1703±73.5	1245±120 c	1530±63.5	1690±125 d
Apo B (mg/dl.hrs) IAUC	248±107	93.3±38.7	115±31.9	269±71.4 d
Apo AI (mg/dl.hrs) AUC	1694±53.4	1767±88.6	1679±67.1	1708±82.5
Apo AI (mg/dl.hrs) IAUC	-31.5±34.4	-83.7±42.7	-133±31.8 d	-193±41.7

Table 4.14. AUC and IAUC for Apo B and Apo AI in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 12 hours.

c: $p<0.001$ compared with start.

d: $p<0.05$ compared with bezafibrate group.

Beza: bezafibrate

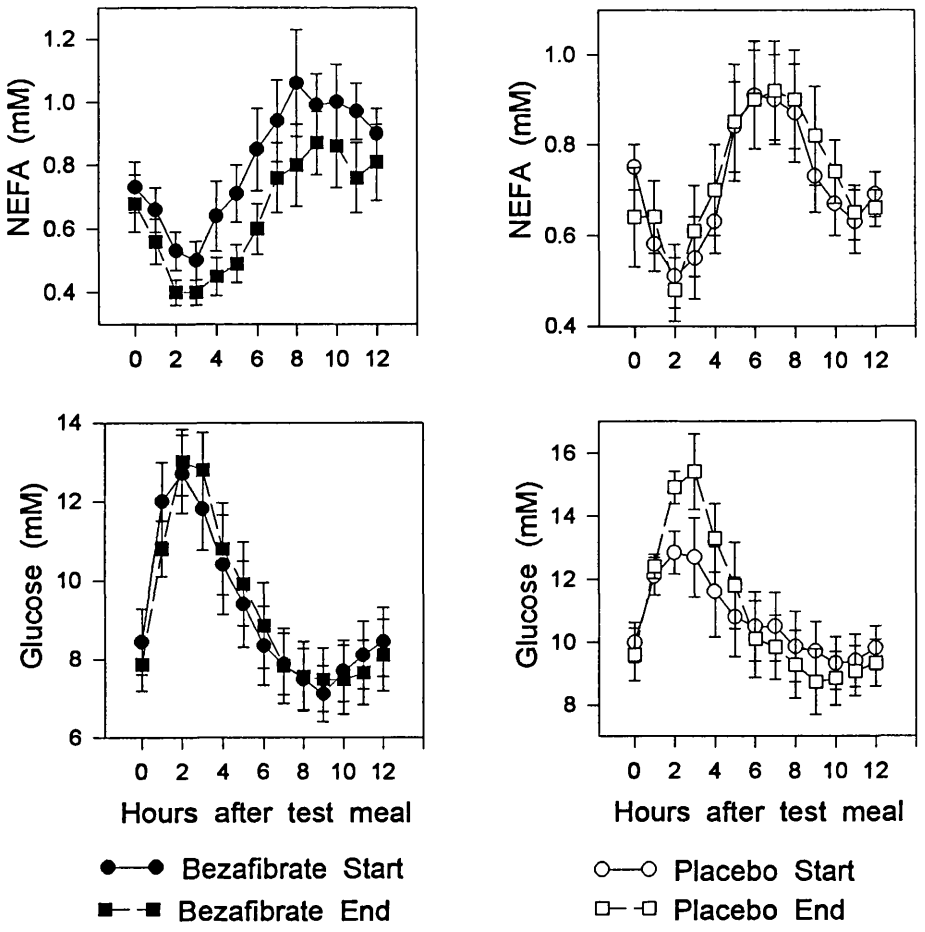


Fig. 4.8. Fasting and postprandial concentrations of NEFA and Glucose in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
NEFA (mM.hrs) AUC	9.68±0.89	7.72±0.82 a	8.54±0.56	8.86±0.70
NEFA (mM.hrs) IAUC	0.96±1.20	-0.44±1.36	-0.51±0.50	1.13±1.31
Glucose (mM.hrs) AUC	111±10.4	112±10.0	129±11.0	133±9.71
Glucose (mM.hrs) IAUC	10.1±4.81	17.7±5.57 a	8.86±5.66	17.8±12.6

Table 4.15. AUC and IAUC for NEFA and glucose in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 12 hours.

a: $p<0.05$ compared with start.

Beza: bezafibrate

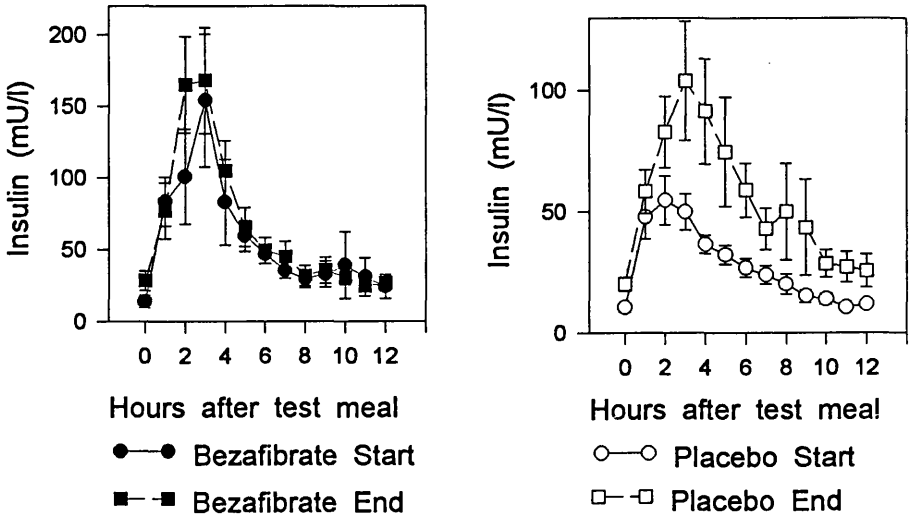


Fig. 4.9. Fasting and postprandial concentrations of insulin in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
Insulin (mU/l.hrs) AUC	717±163	826±138	343±43.8 d	685±144 b
Insulin (mU/l.hrs) IAUC	548±119	483±110	217±36.8 d	443±131 a

Table 4.16. AUC and IAUC for insulin in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 12 hours.
a: $p<0.05$; b: $p<0.01$ compared with start.
d: $p<0.05$ compared with bezafibrate group.
Beza: bezafibrate

4.3.4. Postprandial chylomicrons and VLDL and LDL subfractions in bezafibrate and placebo groups

Postprandial responses for chylomicrons, VLDL subfractions and LDL subfractions are shown in Figs. 4.10-4.13, and the calculated areas under the postprandial curves (AUC) together with the incremental areas under the curves (IAUC) are shown in Tables 4.17-4.20 respectively. Levels of lipid and protein components for these parameters at 3 and 8 hours are shown in Tables 4.22-4.23 and 4.26-4.27 respectively.

AUC for chylomicrons and VLDL-1, -2 and -3, and IAUC for chylomicrons decreased significantly in bezafibrate-treated patients by 55%, 49%, 36%, 27% and 56% respectively (Tables 4.17 and 4.18). Also, AUC for chylomicrons and IAUC for chylomicrons and VLDL-1 were significantly lower compared to the placebo group (Table 4.17). Levels of VLDL-1 (Fig. 4.10) and VLDL-2 (Fig. 4.11) were lower at 8 hours than

at 3 hours after bezafibrate therapy, whereas before bezafibrate treatment, levels were highest at 8 hours, suggesting that rate of clearance of these fractions was increased. Although there was no significant difference in IAUC for VLDL-3 after bezafibrate treatment (Table 4.18), it can be seen from Fig. 4.11 that concentration of VLDL-3 decreased postprandially before bezafibrate treatment whereas levels increased after bezafibrate treatment. Concentrations (mass) of chylomicrons, VLDL-1 and -2 were significantly reduced at 3 and 8 hours following bezafibrate treatment, whereas levels of VLDL-3 were only significantly reduced at 3 hours (Tables 4.22 and 4.26).

Following bezafibrate therapy, there were no differences in postprandial responses of LDL subfractions (Fig. 4.12-4.13, Tables 4.19-4.20) or in total LDL cholesterol (Table 4.21) . However, as with fasting comparisons, cholesterol ester in LDL-1 decreased at 3 hours and free cholesterol in LDL-2 increased at 3 and 8 hours after bezafibrate treatment (Tables 4.23 and 4.27) and the postprandial LDL subfraction profile became less polydisperse (Figs. 4.12 and 4.13). AUC and IAUC for cholesterol and triglyceride in chylomicrons, VLDL subfractions and LDL subfractions showed similar trends after bezafibrate therapy to that for total mass (Table 4.21).

Composition of chylomicrons, VLDL subfractions and LDL subfractions (expressed as percentage of total mass) at 3 and 8 hours are shown in Tables 4.24-4.25 and 4.28-4.29 respectively. At three hours after the test meal, VLDL-1 and -2 % triglyceride was significantly higher in bezafibrate treated patients, with accompanying lower % cholesterol in these fractions (significant for VLDL-2) (Table 4.24). In addition, % free cholesterol was significantly higher and % esterified cholesterol significantly lower in LDL-3 at three hours following bezafibrate therapy (Table 4.25). Percent esterified cholesterol in LDL-3 was significantly lower after bezafibrate therapy 8 hours after the test meal (Table 4.29).

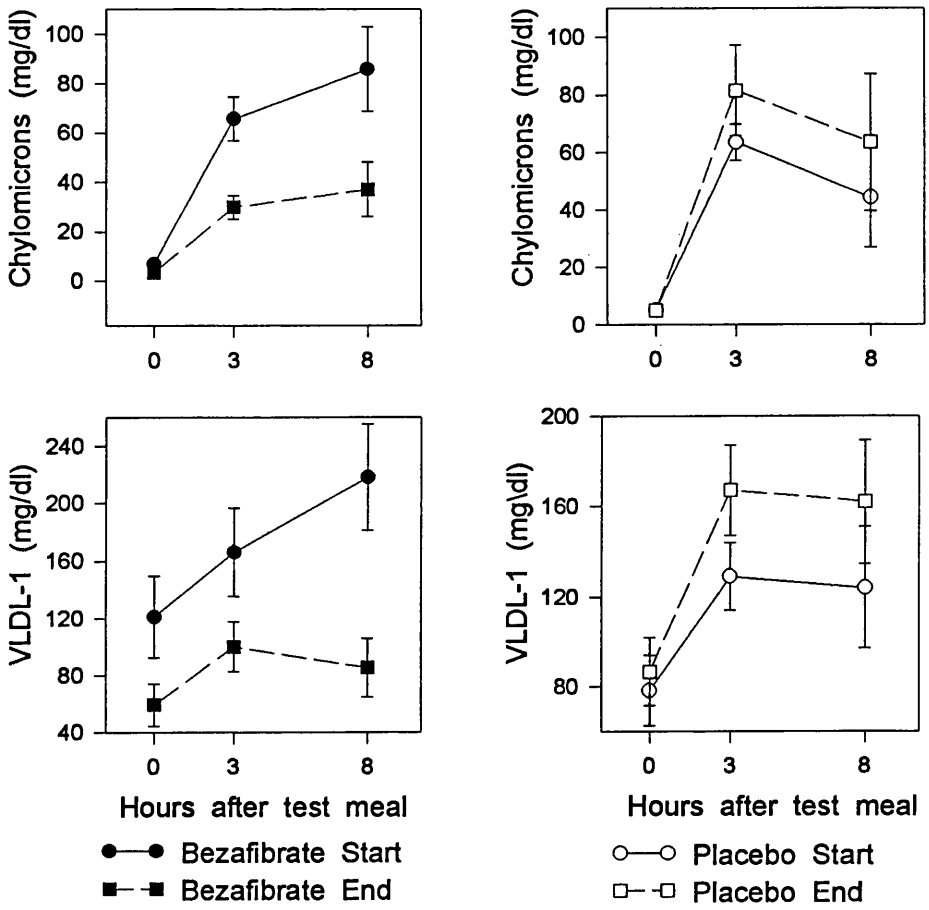


Fig. 4.10. Fasting and postprandial concentrations of chylomicrons and VLDL-1 in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
Chylo (mg/dl.hrs) AUC	487±53.0	217±33.6 b	372±57.0	492±106 e
Chylo (mg/dl.hrs) IAUC	432±51.4	190±30.8 b	332±53.4	453±101 d
VLDL-1 (mg/dl.hrs) AUC	1389±247	702±133 b	944±141	1201±161 a
VLDL-1 (mg/dl.hrs) IAUC	424±62.9	227±79.4	317±52.0	508±86.6 a d

Table 4.17. AUC and IAUC for chylomicrons and VLDL-1 in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 8 hours.
Beza: bezafibrate
a: $p<0.05$; b: $p<0.01$ compared with start.
d: $p<0.05$; e: $p<0.01$ compared with bezafibrate group.

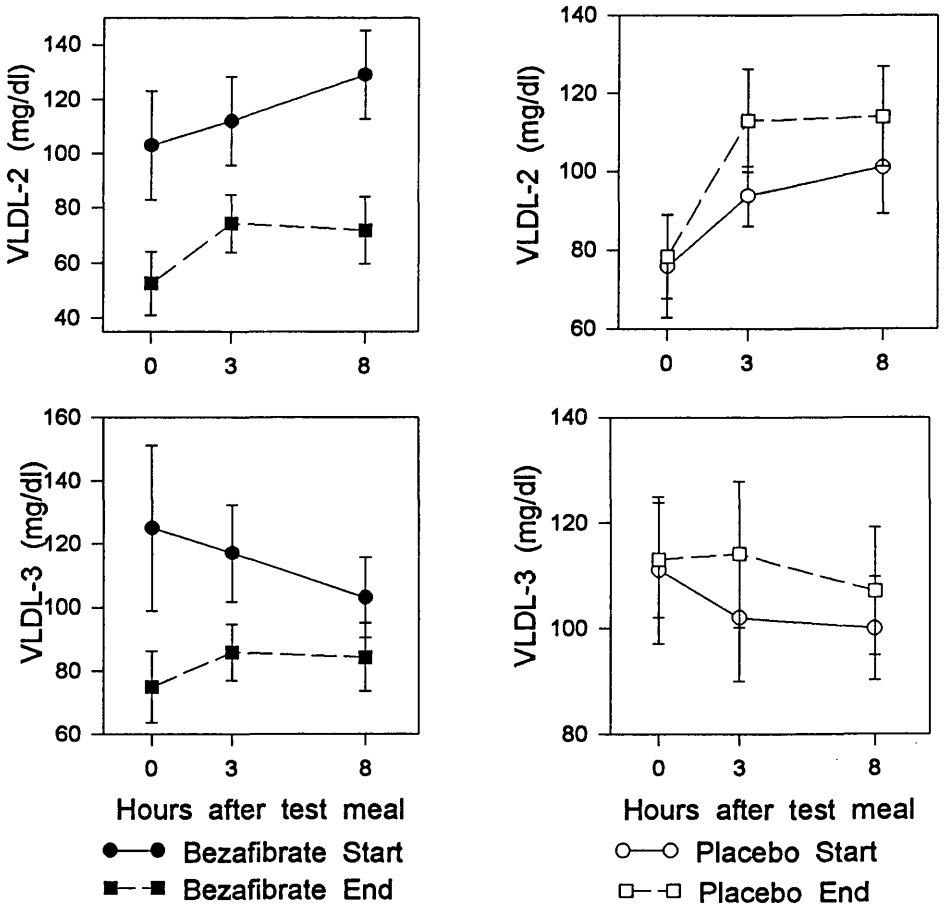


Fig. 4.11. Fasting and postprandial concentrations of VLDL-2 and VLDL-3 in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
VLDL-2 (mg/dl.hrs) AUC	1053±158	673±96.6 b	862±77.2	1018±119
VLDL-2 (mg/dl.hrs) IAUC	231±60.0	253±60.2	256±54.1	392±57.8
VLDL-3 (mg/dl.hrs) AUC	916±129	666±75.8 a	826±86.2	893±97.3
VLDL-3 (mg/dl.hrs) IAUC	-85.1±91.2	67.2±29.1	-60.9±73.2	-10.1±54.5

Table 4.18. AUC and IAUC for chylomicrons and VLDL-1 in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 8 hours.
Beza: bezafibrate
a: $p<0.05$; b: $p<0.01$ compared with start.

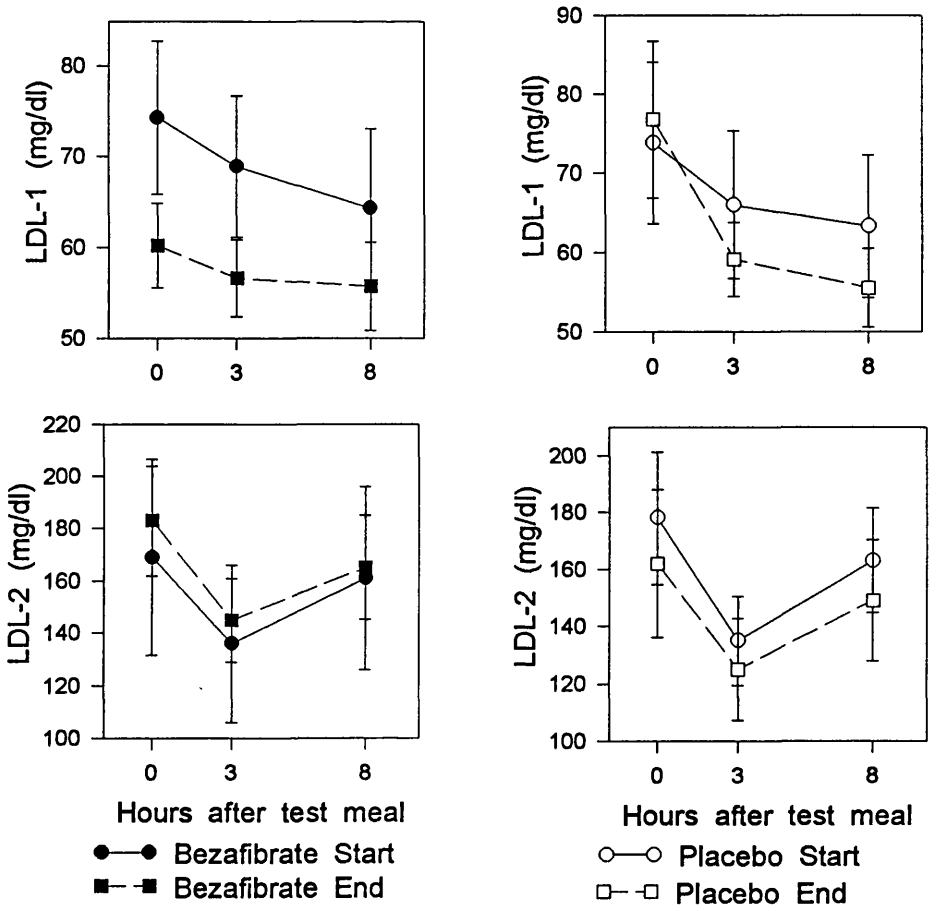


Fig. 4.12. Fasting and postprandial concentrations of LDL-1 and LDL-2 in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
LDL-1 (mg/dl.hrs) AUC	548±63.5	456±34.4	533±74.5	490±41.4
LDL-1 (mg/dl.hrs) IAUC	-46.8±29.3	-25.5±17.0	-57.2±19.5	-124±49.8
LDL-2 (mg/dl.hrs) AUC	1198±262	1266±143	1217±142	1117±162
LDL-2 (mg/dl.hrs) IAUC	-158±46.7	-201±47.1	-206±49.8	-182±56.3

Table 4.19. AUC and IAUC for LDL-1 and LDL-2 in bezafibrate and placebo groups. All values are mean ± SEM and were calculated as a product of concentration and time over 8 hours.
Beza: bezafibrate

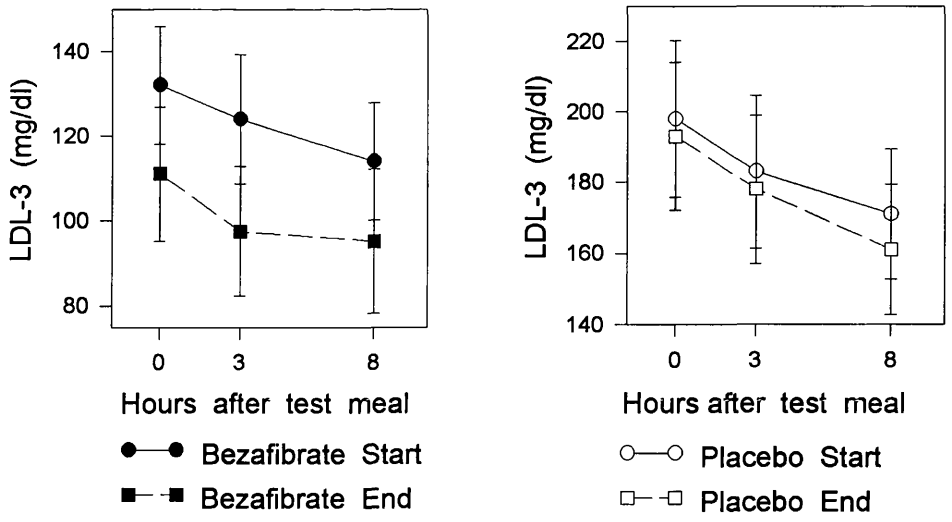


Fig. 4.13. Fasting and postprandial concentrations of LDL-3 in bezafibrate and placebo groups.

	Beza Start	Beza End	Placebo Start	Placebo End
LDL-3 (mg/dl.hrs) AUC	979±115	794±126	1455±165 d	1403±159 e
LDL-3 (mg/dl.hrs) IAUC	-78.7±33.1	-92.8±20.9	-132±28.4	-145±21.9

Table 4.20. AUC and IAUC for LDL-3 in bezafibrate and placebo groups.
All values are mean ± SEM and were calculated as a product of concentration and time over 8 hours.
Beza: bezafibrate
d: $p<0.05$; e: $p<0.01$ compared with bezafibrate group.

Table 4.21. AUC and IAUC for cholesterol and triglyceride in chylomicrons and VLDL and LDL subfractions in bezafibrate- and placebo- treated groups.

	Bezafibrate		Placebo	
	Start	End	Start	End
Chylo chol AUC	21.2±3.94	7.92±1.53 a	11.7±2.80	16.9±2.76 d
Chylo chol IAUC	16.5±4.42	6.38±1.49	8.72±2.69	13.9±2.59 d
VLDL-1 chol AUC	115±24.4	52.5±10.6 a	66.0±9.43	90.2±12.8 ad
VLDL-1 chol IAUC	33.3±9.02	20.1±7.11	21.7±4.94	35.8±6.55 a
VLDL-2 chol AUC	108±16.5	54.3±9.03 b	75.5±7.03	92.9±12.2 d
VLDL-2 chol IAUC	9.85±9.49	13.1±7.25	10.2±7.78	28.5±4.95
VLDL-3 chol AUC	162±27.5	115±14.7	145±16.8	147±15.7
VLDL-3 chol IAUC	-26.9±22.7	8.79±6.68	-22.7±12.1	-13.4±9.95
LDL-1 chol AUC	166±21.3	130±10.1	165±26.5	150±14.9
LDL-1 chol IAUC	-17.8±13.3	-11.0±5.97	-17.4±5.99	-46.9±25.6
LDL-2 chol AUC	419±1.2	418±52.0	443±54.4 d	394±63.4
LDL-2 chol IAUC	-26.3±15.6	-36.0±10.7	-39.0±15.5	-48.8±26.7
LDL-3 chol AUC	306±37.2	241±41.4	476±54.6	445±52.1 e
LDL-3 chol IAUC	-34.5±10.6	-38.2±8.20	-36.9±7.47	-56.8±8.80
Total LDL chol AUC	891±108	789±58.3	1084±101	989±95.2
Total LDL chol IAUC	-78.9±33.3	-85.2±15.8	-93.3±24.5	-152±49.8
Chylo trig AUC	383±37.0	176±26.6 b	309±50.3	403±86.6 e
Chylo trig IAUC	343±33.9	158±24.6 b	280±46.4	377±82.8 d
VLDL-1 trig AUC	921±166	482±89.7 b	638±99.8	806±109
VLDL-1 trig IAUC	275±44.1	147±55.1	222±34.9	347±71.9 d
VLDL-2 trig AUC	515±74.0	332±49.8 b	435±43.3	476±56.9
VLDL-2 trig IAUC	58.4±32.4	74.1±28.3	91.5±35.8	118±20.9
VLDL-3 trig AUC	371±44.1	280±31.7 a	344±37.8	383±45.7
VLDL-3 trig IAUC	-23.1±32.9	44.6±15.8	-11.6±42.8	19.5±28.7
LDL-1 trig AUC	79.8±8.61	74.9±6.22	73.7±7.84	73.5±7.42
LDL-1 trig IAUC	-8.00±4.45	-0.95±4.29	-6.97±5.19	-12.6±5.56
LDL-2 trig AUC	88.4±12.8	94.5±11.4	66.2±7.36	73.2±5.94
LDL-2 trig IAUC	4.24±2.88	-1.97±3.13	-3.73±3.43	-3.80±5.34
LDL-3 trig AUC	53.7±6.92	43.7±6.45	47.8±7.88	58.0±4.50
LDL-3 trig IAUC	3.52±2.95	3.52±2.74	-0.66±2.81	2.75±2.45
Total LDL trig AUC	222±21.6	213±19.0	188±18.2	205±12.3
Total LDL trig IAUC	-0.24±7.13	0.59±6.78	-11.4±10.4	-13.6±7.19

All areas were calculated as a product of concentration (mg/dl) and time (hours) over 8 hours. All values are mean ± SEM mg/dl.hours.

a: $p<0.05$; b: $p<0.01$ compared with start.

d: $p<0.05$; e: $p<0.01$ compared with bezafibrate group.

Table 4.22. Concentrations of lipid and protein components of chylomicrons and VLDL subfractions in bezafibrate and placebo groups 3 hours after test meal.

	Chylomicrons				VLDL-1				VLDL-2				VLDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	2.29± 0.36	1.15± 0.29 a	1.68± 0.39	2.53± 0.54 d	13.2± 2.94	7.25± 1.56	8.55± 0.96	12.4± 1.61 ad	13.3± 2.03	6.71± 1.31 a	9.13± 0.75	12.4± 1.81 d	20.8± 3.01	14.7± 1.67	17.8± 2.23	18.8± 2.21
TG	53.9± 7.25	24.7± 4.07 b	53.7± 5.40	68.0± 12.6 f	110± 20.3	69.6± 11.8 a	88.8± 10.5	112± 13.9	61.6± 9.22	44.7± 6.00 a	56.0± 4.76	62.2± 7.89	47.2± 5.28	36.6± 3.74 a	42.2± 5.78	49.2± 6.59
PL	4.01± 0.70	1.66± 0.37 a	3.50± 0.72	5.00± 1.20 d	21.3± 4.41	1.80± 2.38 a	15.5± 2.02	21.4± 2.64 bd	18.4± 2.97	11.3± 1.84 a	13.7± 1.49	20.3± 2.90 ad	22.3± 3.36	15.4± 1.75	19.0± 1.87	21.3± 2.86
FC	0.89± 0.21	0.40± 0.14 a	0.89± 0.23	1.30± 0.36 d	6.53± 1.31	3.77± 0.88 a	5.03± 0.74	6.39± 1.02	5.94± 1.01	3.22± 0.75 a	4.71± 0.50	5.97± 0.98 d	8.03± 1.25	6.53± 0.65	7.36± 0.74	8.31± 1.18
EC	2.34± 0.36	1.25± 0.27 a	1.33± 0.30 d	2.07± 0.50	11.2± 3.06	5.84± 1.16 b	5.91± 0.60	10.1± 1.31 bd	12.3± 2.11	5.87± 1.03 a	7.43± 0.68 d	10.8± 1.46 ad	21.4± 3.20	13.7± 1.88	17.5± 2.87	17.6± 1.84
Prot	4.56± 0.93	1.92± 0.20 a	4.04± 0.44	5.13± 1.69	17.4± 2.89	9.12± 1.76 a	14.0± 2.02	16.7± 1.88 e	14.1± 2.07	9.17± 1.35 c	11.7± 1.11	13.4± 1.69	18.5± 3.13	13.6± 1.33	16.0± 1.73	17.6± 1.71
Mass	65.7± 8.92	29.9± 4.74 b	63.4± 6.27	85.5± 15.7 e	166± 30.6	100± 17.5 b	129± 14.9	167± 20.1 ad	112± 16.3	74.3± 10.4 b	93.6± 7.65	113± 13.2	117± 15.3	85.8± 8.89 a	102± 12.0	114± 13.8

All values are mean ± SEM (mg/dl).

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared with start.

d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared with bezafibrate group.

Table 4.23. Concentrations of lipid and protein components of LDL subfractions in bezafibrate and placebo groups 3 hours after test meal.

	LDL-1				LDL-2				LDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	20.8± 2.66	16.0± 1.23	20.6± 3.28	17.9± 1.61	52.0± 12.6	51.7± 6.17	54.7± 6.76	48.6± 7.90	38.3± 4.80	29.3± 4.99	60.0± 7.33 d	56.4± 6.59 d
TG	9.87± 1.05	9.33± 0.81	9.24± 1.04	8.97± 0.79	10.9± 1.50	11.6± 1.41	8.25± 0.89	8.69± 0.76	6.73± 0.87	5.48± 0.89	6.04± 1.00	7.21± 0.70
PL	14.5± 1.60	12.0± 1.26	13.6± 1.80	12.3± 1.29	33.4± 7.55	36.7± 4.14	34.0± 3.93	32.0± 4.57	21.9± 2.69	19.2± 3.04	34.5± 4.09 d	33.9± 4.22 d
FC	5.76± 0.77	5.18± 0.52	6.09± 0.78	5.51± 0.58	13.5± 3.58	16.9± 2.68 a	14.3± 1.73	13.4± 1.70	7.47± 1.05	8.53± 1.67	13.8± 1.55 e	12.7± 1.36
EC	25.2± 3.57	18.2± 1.53 a	24.3± 4.29	20.9± 1.83	36.8± 9.19	33.1± 3.84	38.7± 5.16	33.51± 6.51	51.8± 6.40	34.8± 6.38	77.8± 9.85 d	73.4± 8.95 d
Prot	13.6± 2.05	11.9± 0.89	12.7± 2.12	12.5± 1.02	10.9± 9.18	46.1± 5.97	40.3± 5.44	37.8± 5.69	35.9± 4.98	29.4± 4.20	50.5± 6.01	50.6± 6.11 d
Mass	68.9± 7.76	56.6± 4.26	66.0± 9.34	59.1± 4.66	136± 30.0	145± 16.0	135± 15.5	125± 17.8	124± 15.3	97.5± 15.2	183± 21.6	178± 20.7 d

All values are mean ± SEM (mg/dl).

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$ compared with start.

d: $p<0.05$; e: $p<0.01$ compared with bezafibrate group.

Table 4.24. Composition of chylomicrons and VLDL subfractions in bezafibrate and placebo groups 3 hours after test meal.

	Chylomicrons				VLDL-1				VLDL-2				VLDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	3.40± 0.18	4.00± 0.63	2.54± 0.54	2.99± 0.42	7.92± 0.58	6.66± 0.73	6.60± 0.39	7.35± 0.26	11.8± 0.43	8.62± 1.06 a	9.83± 0.43 e	10.7± 0.72	17.6± 0.56	17.0± 0.48	17.4± 0.50	16.5± 0.54
TG	82.4± 1.04	81.3± 1.97	84.5± 1.31	84.3± 1.11	66.1± 0.97	70.7± 1.44 b	68.7± 1.06	67.4± 1.14	54.7± 1.27	61.5± 1.56 b	59.8± 0.97 e	55.6± 2.31	40.7± 1.15	43.1± 1.41	40.0± 2.61	42.9± 1.13
PL	6.00± 0.35	5.55± 0.60	5.56± 0.85	5.63± 0.75	11.9± 0.96	11.2± 0.91	12.0± 0.75	12.8± 0.54	16.0± 0.99	14.5± 0.79	14.6± 0.88	17.9± 1.90	18.8± 0.59	17.8± 0.47	19.8± 2.28	18.4± 0.50
FC	1.26± 0.20	1.19± 0.30	1.32± 0.32	1.44± 0.22	3.68± 0.34	3.22± 0.52	3.62± 0.48	3.70± 0.35	4.95± 0.68	3.87± 0.73	4.97± 0.42	5.09± 0.50	6.75± 0.40	7.84± 0.55	7.60± 0.79	7.17± 0.33
EC	3.60± 0.37	4.72± 0.90	2.04± 0.43 d	2.59± 0.50	7.12± 1.35	5.78± 0.51	5.01± 0.60	6.13± 0.36	11.5± 1.59	7.98± 0.79	8.17± 0.67	9.46± 0.58	18.2± 1.21	15.33± 1.17	16.4± 1.63	15.7± 0.85
Prot	6.71± 0.95	7.23± 0.97	6.59± 0.71	6.00± 0.75	11.1± 1.81	9.10± 0.55	10.6± 0.69	10.0± 0.59	12.9± 1.51	12.2± 0.20	12.5± 0.59	11.9± 0.83	15.5± 1.71	15.9± 0.60	16.1± 0.93	15.8± 0.60

All values are mean % of total mass ± SEM.

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$ compared with start.

d: $p<0.05$; e: $p<0.01$ compared with bezafibrate group.

Table 4.25. Composition of LDL subfractions in bezafibrate and placebo groups 3 hours after test meal.

	LDL-1				LDL-2				LDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	29.9± 0.88	28.3± 0.51	30.8± 1.02	30.2± 1.06	37.1± 1.56	36.1± 1.71	40.1± 1.23	38.4± 1.60	31.0± 0.53	29.3± 0.97	32.8± 0.35 d	31.7± 0.31 d
TG	14.8± 1.31	16.5± 0.93	14.7± 1.52	15.7± 1.35	9.59± 1.12	8.07± 0.62	6.60± 0.81	7.63± 0.87	5.60± 0.47	5.98± 0.54	3.32± 0.46 e	4.28± 0.41 d
PL	21.1± 0.66	20.9± 1.11	20.8± 0.38	20.6± 0.92	24.3± 0.75	25.5± 1.00	25.2± 0.85	25.6± 1.11	17.8± 0.64	19.6± 0.96	19.0± 0.35	19.0± 0.41
FC	8.35± 0.56	9.11± 0.72	9.41± 0.40	9.21± 0.38	9.70± 0.54	11.4± 0.63	10.5± 0.37	10.8± 0.35	5.96± 0.37	8.39± 0.95 a	7.64± 0.28 e	7.31± 0.37
EC	36.2± 2.01	32.3± 1.49	35.9± 2.01	35.3± 1.62	25.6± 1.92	23.4± 1.68	28.2± 1.34	26.1± 1.93	42.1± 0.65	35.2± 2.43 a	42.2± 0.72	41.0± 0.87 d
Prot	19.5± 1.83	21.1± 1.02	19.1± 0.73	19.2± 2.09	30.8± 1.85	31.6± 1.30	29.5± 1.54	29.9± 0.85	28.6± 1.09	30.8± 1.29	27.9± 0.80	28.4± 0.44

All values are mean % of total mass ± SEM.

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$ compared with start.

d: $p<0.05$; e: $p<0.01$ compared with bezafibrate group.

Table 4.26. Concentrations of lipid and protein components of chylomicrons and VLDL subfractions in bezafibrate and placebo groups 8 hours after test meal.

	Chylomicrons				VLDL-1				VLDL-2				VLDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	4.47± 1.18	1.21± 0.30 a	1.75± 0.65	2.47± 0.75	18.6± 4.02	6.96± 1.54 a	9.39± 2.02	12.1± 2.00 d	14.7± 1.95	7.88± 1.41 b	10.7± 1.18	12.5± 1.39 d	17.5± 2.75	14.5± 2.00	16.9± 2.02	16.7± 1.84
TG	64.2± 14.0	29.4± 8.82 a	35.4± 15.2 d	50.2± 19.4	145± 25.4	56.5± 13.9 b	81.9± 19.0	109± 19.1 d	73.3± 9.75	41.7± 6.94 a	58.5± 7.28	64.0± 7.76	43.4± 4.64	35.9± 4.50	43.3± 4.20	47.2± 5.28
PL	8.42± 2.39	1.93± 0.83 a	3.19± 1.50	4.78± 2.15	25.8± 4.74	10.5± 2.35 b	15.9± 3.14	20.4± 3.68 bd	20.2± 2.53	10.8± 2.11 b	16.0± 1.82	18.5± 2.14 d	19.4± 2.31	15.5± 2.39	19.0± 1.66	20.3± 2.67
FC	2.15± 0.86	0.50± 0.28	1.02± 0.62	1.32± 0.58	8.28± 1.81	3.48± 0.80 b	4.92± 1.06	6.54± 1.40 a	6.25± 0.85	3.55± 0.69 c	5.20± 0.66	5.89± 0.77 d	6.69± 0.94	6.12± 0.90	7.19± 0.63	7.55± 0.99
EC	3.90± 1.09	1.20± 0.26	1.23± 0.32 d	1.94± 0.37 a	17.3± 4.71	5.86± 1.28 a	7.50± 1.86	9.34± 1.48	14.2± 2.33	7.28± 1.23 a	9.21± 1.05	11.2± 1.19 d	18.1± 3.30	14.0± 1.86	16.4± 2.54	15.4± 1.58
Prot	6.93± 1.89	3.94± 1.61	3.40± 0.77	5.14± 2.02	21.5± 3.58	8.71± 2.91 b	13.5± 2.69	16.7± 3.01	15.4± 2.33	8.43± 1.38 b	11.6± 1.56	14.4± 1.69 d	15.7± 2.11	12.8± 1.59	14.6± 1.44	16.6± 2.20
Mass	85.7± 17.1	37.0± 10.9 a	44.2± 17.4 d	63.4± 23.9	21.8± 37.1	85.1± 20.4 b	124± 27.0	162± 27.5 d	129± 16.3	71.8± 12.2 a	101± 11.8	114± 12.8	103± 12.5	84.2± 10.8	100± 9.71	107± 12.1

All values are mean ± SEM (mg/dl).

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared with start.

d: $p<0.05$ compared with bezafibrate group.

Table 4.27. Concentrations of lipid and protein components of LDL subfractions in bezafibrate and placebo groups 8 hours after test meal.

	LDL-1				LDL-2				LDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	19.4± 2.96	15.9± 1.49	19.5± 3.29	16.4± 1.60	51.1± 12.5	50.5± 6.98	53.5± 6.11	46.6± 7.08	35.5± 4.42	28.5± 5.63	55.9± 5.96 d	50.0± 6.26 ad
TG	9.54± 1.17	9.35± 0.96	8.64± 0.99	8.58± 1.20	11.6± 1.87	12.0± 1.64	8.05± 0.84	9.61± 0.93	6.93± 1.04	5.70± 0.89	5.82± 0.88	7.53± 0.55
PL	13.4± 1.70	10.9± 1.35	12.7± 1.83	11.3± 1.40	32.7± 7.74	35.5± 4.49	31.8± 3.50	30.5± 4.43	20.0± 2.35	18.1± 3.16	32.8± 3.28 e	29.6± 4.05 d
FC	5.39± 0.83	4.93± 0.49	5.99± 0.84	5.02± 0.64	13.2± 3.71	16.6± 3.08 b	13.1± 1.47	12.7± 1.62	6.81± 0.96	8.14± 1.33	12.9± 1.23 e	11.0± 1.21 a
EC	23.5± 2.89	18.4± 1.92	22.6± 4.21	19.1± 1.87	63.6± 15.1	56.8± 7.07	68.0± 8.0	57.0± 9.34	48.2± 6.00	34.2± 8.42	72.2± 8.11 d	65.4± 8.61 d
Prot	12.3± 1.94	12.2± 1.05	13.4± 1.80	11.5± 1.01	39.4± 7.23	44.4± 5.46	42.4± 6.27	38.8± 5.72	32.2± 4.23	29.0± 4.46	47.1± 6.04	47.0± 4.85 d
Mass	64.3± 8.78	55.7± 4.87	63.3± 8.99	55.5± 4.95	161± 34.6	165± 19.8	163± 18.4	149± 21.2	114± 13.8	95.2± 16.9	171± 18.3 d	161± 18.4 d

All values are mean ± SEM (mg/dl).

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$ compared with start.

d: $p<0.05$; e: $p<0.01$ compared with bezafibrate group.

Table 4.28. Composition of chylomicrons and VLDL subfractions in bezafibrate and placebo groups 8 hours after test meal.

	Chylomicrons				VLDL-1				VLDL-2				VLDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	5.65± 1.43	4.11± 0.70	4.71± 1.21	5.55± 0.87	8.14± 0.63	8.42± 0.89	7.85± 0.73	7.40± 0.32	11.4± 0.39	10.5± 0.45	10.8± 0.47	11.1± 0.46	16.6± 0.62	17.1± 0.49	16.7± 0.71	15.7± 0.58 b
TG	75.6± 5.58	78.5± 3.92	72.9± 7.50	77.1± 2.68	66.0± 1.21	66.4± 3.13	64.7± 2.08	67.6± 1.20	56.3± 1.19	59.1± 1.02	57.6± 1.32	56.1± 1.31	42.4± 1.31	42.6± 1.22	43.1± 1.11	44.2± 1.01
PL	9.67± 2.74	4.73± 0.63	7.24± 1.56	7.27± 1.14	11.6± 0.40	12.1± 0.75	13.6± 0.94	12.5± 0.49	15.5± 0.49	13.8± 1.11	16.2± 0.79	16.3± 0.76	18.8± 0.50	18.1± 0.85	19.2± 0.92	18.7± 0.83
FC	2.60± 0.93	1.23± 0.43	1.67± 0.48	2.25± 0.52	3.61± 0.25	4.21± 0.67	4.04± 0.31	3.82± 0.29	4.80± 0.39	4.28± 0.61	5.19± 0.30	5.08± 0.31	6.48± 0.46	7.20± 0.27	7.25± 0.30	6.97± 0.27
EC	5.13± 1.15	4.84± 1.08	5.11± 1.62	5.53± 0.91	7.61± 1.20	7.07± 1.10	6.39± 0.98	6.01± 0.56	11.1± 1.25	10.5± 0.37	9.44± 0.76	10.0± 0.78	16.9± 1.42	16.7± 0.46	15.9± 1.25	14.6± 0.87
Prot	7.00± 1.43	10.7± 3.48	13.1± 4.70	7.87± 0.97	11.2± 2.02	10.2± 1.85	11.4± 0.70	10.0± 0.90	12.3± 1.85	12.4± 1.07	11.5± 0.87	12.4± 0.62	15.4± 1.31	15.4± 0.86	14.6± 0.47	15.5± 0.77

All values are mean % of total mass ± SEM.

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

b: $p<0.01$ compared with start.

Table 4.29. Composition of LDL subfractions in bezafibrate and placebo groups 8 hours after test meal.

	LDL-1				LDL-2				LDL-3			
	Bezafibrate		Placebo		Bezafibrate		Placebo		Bezafibrate		Placebo	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
CH	29.9± 0.63	28.4± 0.72	30.1± 1.21	29.5± 1.05	30.7± 1.03	30.4± 1.10	32.7± 0.71	31.2± 0.77	31.0± 0.43	29.1± 1.21	32.9± 0.58 d	30.8± 0.51 a
TG	15.4± 1.27	17.0± 1.37	14.3± 1.45	15.6± 1.48	8.25± 0.88	7.29± 0.64	5.28± 0.62 d	7.01± 0.71 a	6.25± 0.67	6.73± 0.97	3.41± 0.42 e	5.19± 0.71
PL	21.1± 0.71	19.2± 1.02	20.0± 0.69	19.9± 1.05	19.9± 0.74	21.4± 0.87	19.5± 0.54	20.5± 0.67	17.8± 0.84	19.1± 1.07	19.5± 0.60	18.1± 0.66
FC	8.44± 0.80	8.88± 0.48	9.50± 0.43	8.73± 0.57	7.95± 0.58	9.71± 0.69	8.08± 0.45	8.71± 0.24	5.86± 0.47	9.68± 2.31	7.75± 0.37 e	6.97± 0.27
EC	36.0± 1.90	32.7± 1.49	34.6± 2.07	34.8± 1.97	32.3± 1.72	34.7± 1.90	41.4± 1.06	37.7± 1.58	42.2± 0.58	32.6± 3.54 a	42.2± 0.70	40.1± 1.13
Prot	19.0± 1.39	22.2± 1.54	21.6± 1.10	21.0± 0.90	25.7± 1.97	26.9± 0.97	25.7± 1.47	26.0± 0.73	27.9± 1.00	31.9± 1.20	17.1± 1.20	29.6± 0.81

All values are mean % of total mass ± SEM.

CH: cholesterol; TG: triglyceride; PL: phospholipid; FC: free cholesterol; EC: esterified cholesterol; Prot: protein.

a: $p<0.05$ compared with start.

d: $p<0.05$; e: $p<0.01$ compared with bezafibrate group.

Qualitative lipoprotein subfraction parameters at 3 and 8 hours after the test meal are shown in Tables 4.30 and 4.31 respectively. At 3 and 8 hours after the test meal, %LDL-3/∑LDL was significantly lower after bezafibrate treatment, and was also significantly lower than the placebo group. At 8 hours only, % VLDL-1/∑VLDL was significantly lower after bezafibrate treatment, and was also significantly lower than the placebo group, and % VLDL-3/∑VLDL was significantly elevated after bezafibrate treatment, indicating an improved clearance rate of large VLDL. This is further emphasised by comparing paired analyses of fasting versus postprandial percentages within patients before and after bezafibrate treatment (Table 4.32): % VLDL-1/∑VLDL was significantly higher at 3 and 8 hours before bezafibrate therapy, whereas after bezafibrate therapy levels were only significantly elevated at 3 hours. Also, % VLDL-3/∑VLDL was significantly reduced at 3 and 8 hours before bezafibrate therapy, whereas after bezafibrate therapy levels were only significantly lower at 3 hours. No such trends were observed in the placebo group (Table 4.33).

Similarly to fasting differences, bezafibrate therapy did not significantly alter HDL_2/HDL_3 and LDL/HDL cholesterol ratios at 3 and 8 hours. There was a significant reduction in HDL and HDL_3 triglyceride enrichment (triglyceride/cholesterol ratio) at 8 hours in bezafibrate treated patients.

Table 4.30. Qualitative parameters in bezafibrate- and placebo groups 3 hours after the test meal.

	Bezafibrate		Placebo	
	Start	end	Start	end
%VLDL-1/ ∑VLDL	39.0±3.60	35.7±2.86	39.0±2.93	41.9±1.86
%VLDL-2/ ∑VLDL	28.1±0.53	27.9±1.22	28.9±0.57	29.7±0.71
%VLDL-3/ ∑VLDL	32.9±3.77	36.4±3.60	32.1±3.19	29.5±2.15
%LDL-3/∑LDL	39.9±5.16	31.9±4.47 a	47.2±3.20	49.1±3.40 a e
LDL chol/ HDL chol	3.19±0.29	2.99±0.19	3.85±0.39	3.43±0.22
HDL_2/ HDL_3 chol	0.80±0.20	0.96±0.28	0.54±0.09	0.46±0.13
HDL trig/chol	0.36±0.03	0.31±0.04	0.25±0.05 d	0.31±0.05
HDL_2 trig/chol	0.32±0.05	0.31±0.09	0.23±0.07	0.32±0.04
HDL_3 trig/chol	0.42±0.03	0.36±0.06	0.28±0.05 d	0.33±0.05

All values are mean ± SEM .
Chol: Cholesterol; Trig: Triglyceride
a: $p<0.05$ compared with start.
d: $p<0.05$; e: $p<0.01$ compared with bezafibrate group.

Table 4.31: Qualitative parameters in bezafibrate- and placebo groups 8 hours after the test meal.

	Bezafibrate		Placebo	
	Start	end	Start	end
%VLDL-1/ ΣVLDL	45.6±3.96	31.9±3.55 b	35.2±4.19	40.4±3.07 a
%VLDL-2/ ΣVLDL	28.4±1.10	28.1±2.25	31.0±1.03	29.7±0.71
%VLDL-3/ ΣVLDL	26.0±3.98	40.0±4.36 b	33.8±4.09	29.9±3.45
%LDL-3/ΣLDL	36.5±5.10	29.6±5.05 a	43.1±3.13	44.5±3.33 d
LDL chol/ HDL chol	3.64±0.39	3.00±0.22	4.12±0.44	3.58±0.26
HDL_2/ HDL_3 chol	0.90±0.28	0.75±0.23	0.95±0.32	0.74±0.34
HDL trig/chol	0.53±0.08	0.38±0.06 a	0.29±0.05 d	0.33±0.05
HDL_2 trig/chol	0.34±0.04	0.61±0.31	0.19±0.04 d	0.50±0.22
HDL_3 trig/chol	0.66±0.12	0.47±0.10 a	0.44±0.06	0.37±0.09

All values are mean ± SEM. Chol: Cholesterol; Trig: Triglyceride.
a: $p<0.05$; b: $p<0.01$ compared with start; d: $p<0.05$ compared with bezafibrate group.

Table 4.32. Comparison of paired data (0hrs vs 3hrs and 0hrs vs 8hrs) within each subject group.

	Bezafibrate group					
	Start			End		
	0 hours	3 hours	8 hours	0 hours	3 hours	8 hours
%VLDL-1/ ΣVLDL	32.0± 4.23	39.0± 3.60 a	45.6± 3.96 c	27.7± 3.18	35.7± 2.86 a	31.9± 3.55
%VLDL-2/ ΣVLDL	28.7± 1.07	28.1± 0.53	28.4± 1.10	25.9± 1.66	27.9± 1.22	28.1± 2.25
%VLDL-3/ ΣVLDL	39.4± 1.70	32.9± 3.77 a	26.0± 3.98 c	46.3± 4.49	36.4± 3.60 a	40.0± 4.36

All values are mean ± SEM.
Fasting levels vs levels at 3 and 8 hours. a: $p<0.05$; c: $p<0.001$

Table 4.33. Comparison of paired data (0hrs vs 3hrs and 0hrs vs 8hrs) within the placebo group at the start and end of the study.

	Placebo group					
	Start			End		
	0 hours	3 hours	8 hours	0 hours	3 hours	8 hours
%VLDL-1/ ΣVLDL	26.1± 3.44	39.0± 2.93 a	35.2± 4.19	29.0± 2.50	41.9± 1.86 b	40.2± 3.07 a
%VLDL-2/ ΣVLDL	27.3± 1.89	28.9± 0.57	31.0± 1.03	27.9± 1.18	28.6± 1.11	29.7± 0.71
%VLDL-3/ ΣVLDL	46.6± 4.86	32.1± 3.19 c	33.8± 4.08 b	43.1± 2.96	29.5± 2.15 c	29.9± 3.45 b

All values are mean ± SEM.
Fasting levels vs levels at 3 and 8 hours. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

4.4. DISCUSSION

This study demonstrates that bezafibrate significantly reduces fasting and postprandial levels of triglyceride-rich lipoproteins and small dense LDL in patients with NIDDM. Several studies have reported that bezafibrate therapy effectively reduces serum triglyceride concentrations in NIDDM (Seviour et al., 1988; Niort et al., 1992; Rovellini et al., 1992). Reductions in postprandial triglyceride response and chylomicron remnants have been demonstrated in NIDDM following gemfibrozil treatment (Syvanne et al., 1993).

Reductions in levels of serum triglyceride, triglyceride-rich lipoproteins and triglyceride-rich lipoprotein subfractions were on the whole more highly significant in the postprandial state. Fasting and postprandial chylomicrons and VLDL-1 were more substantially reduced than the smaller VLDL subfractions. Additionally, the proportion of large VLDL (VLDL-1) was reduced following bezafibrate therapy, particularly in the postprandial state and was reflected by an increase in the proportion of VLDL-3. In this way, the VLDL subfraction profile was reversed towards that of non-diabetic normolipidaemic population (see chapter 3). Furthermore, the postprandial trend in VLDL-3 levels (Fig. 11) before and after bezafibrate treatment resembles that of the diabetic patients and non-diabetic controls (in chapter three) respectively. As discussed in chapter three, it appears that in diabetic patients (before therapy), the production by the liver of small VLDL-3 has been reduced in favour of larger triglyceride-rich VLDL. This would occur when the triglyceride accumulation compared to apo B synthesis is very high and in this way more triglyceride has to be packaged into each VLDL particle. Reduced levels of small VLDL could also arise from slower degradation of large VLDL and chylomicron remnants. In patients after bezafibrate therapy and in the controls in chapter three, the accumulation of triglyceride is less allowing a greater proportion of small VLDL to be produced.

Although concentrations of triglyceride-rich lipoproteins were substantially reduced, often as much as 50%, levels were still higher than those of the non-diabetic normolipidaemic controls in chapter 3, suggesting that bezafibrate treatment was not sufficient to achieve full normalisation. An exception to the aforementioned was AUC for chylomicrons which was reduced by bezafibrate treatment to levels below that of the non-diabetic controls in chapter three, thus further emphasising the efficacy of the drug on the larger triglyceride-rich lipoproteins, particularly in the postprandial state. Reductions in VLDL triglyceride of similar magnitude have been reported in NIDDM patients with hyperlipidaemia (-56%) (Niort et al., 1993) and non-diabetic patients with hyperlipidaemia

(-66%) (Homma et al., 1994), following bezafibrate therapy. Furthermore, reductions in cholesterol and triglyceride in large VLDL after bezafibrate therapy has been demonstrated to be more apparent than in small VLDL in IDDM patients with combined hyperlipidaemia (Winocour et al., 1992).

It is recognised that the hypotriglyceridaemic effect of bezafibrate is mainly mediated by stimulus of LPL and to some extent HL (Vessby et al., 1980; Vessby et al., 1982; Heller et al., 1983; Eisenberg et al., 1984) leading to accelerated degradation of VLDL. Such an effect is consistent with the reduction of triglyceride-rich lipoproteins in the present study. An increase in the fractional catabolic rate of triglyceride-rich particles has been reported in patients with NIDDM following bezafibrate therapy (Seviour et al., 1988). Reduction in VLDL, especially the large triglyceride-rich species, decreases their atherogenic potential because large VLDL is less readily converted to LDL (Stalenhoef et al., 1984; Packard et al., 1984), and less available to provide surface components for HDL. In addition, triglyceride-rich VLDL are susceptible to cholesterol ester transfer from HDL and LDL in exchange for triglyceride by the actions of CETP (Tall, 1986). Triglyceride-rich LDL becomes substrate for lipases resulting in the production of small dense LDL (Auwerx et al., 1988; Levy et al., 1990; Zambon et al., 1993). VLDL becomes cholesterol ester enriched, a modification that provokes clearance via the scavenger pathway (Goldstein, 1980). Kinetic studies of LDL metabolism suggest that small dense LDL (LDL-3) are derived from large triglyceride-rich VLDL in the hypertriglyceridaemic state, whereas in the normotriglyceridaemic state, the liver produces small VLDL particles which give rise to larger, more buoyant LDL (LDL-1 and -2) (Caslake et al., 1992).

Percentage esterified cholesterol (in terms of total mass) was reduced and % triglyceride increased, in fasting VLDL-1 and -2, and % cholesterol reduced and % triglyceride increased in postprandial VLDL-2 after bezafibrate treatment. Since these VLDL subfractions were not triglyceride-enriched following treatment (as denoted by triglyceride/apo B ratios), the increase in % triglyceride is more likely to be a reflection of reduced % cholesterol. This may be the result of decreased residence time of triglyceride-rich lipoproteins in the blood for cholesterol ester transfer with LDL and HDL. Bezafibrate has been reported to reduce the activity of CETP in patients with IDDM (Homma et al., 1994). The size of VLDL particles within each subfraction, as denoted by non-apo B protein/ apoB and triglyceride/apo B ratios, appeared to be unaffected as a result of bezafibrate treatment.

The proportion of small, dense LDL in total LDL mass was significantly reduced after bezafibrate treatment, which is consistent with reductions in triglyceride-rich

lipoproteins, and further evidence of reduced cholesterol ester transfer. This effect of the drug was not apparent from measurements of total and LDL cholesterol. The LDL profile was less polydisperse, with closer resemblance to that of the non-diabetic controls (see chapter three), but the proportion of LDL-3 in total LDL was not normalised to the levels seen in the non-diabetic controls. Previous studies have reported that fibrate therapy reduces the density of LDL in type II hyperlipidaemic subjects (Tsai et al., 1992), in NIDDM (Lahdenpera et al., 1993) and in patients with hyperlipidaemia (Homma et al.,1994), an effect which has great potential in reducing atherogenic risk. The predominance of small, dense LDL is a strong marker for the risk of developing premature CHD in the non-diabetic population (Austin et al., 1988), and in view of their atherogenic potential, small, dense LDL are more readily oxidised (Tribble et al., 1992) and have been shown to bind less well to LDL receptors compared to larger LDL species (Kleinman et al., 1987a; Kleinman et al., 1987b).

Quite surprisingly, there was not a significant fasting or postprandial increase in HDL cholesterol, or decrease in HDL triglyceride following bezafibrate therapy. However, triglyceride/cholesterol ratio in HDL_3 in the postprandial state was significantly decreased suggesting that cholesterol ester transfer with triglyceride lipoproteins was reduced. HDL cholesterol levels have been shown to be increased by fibrates (Rouffy et al., 1985; Sorisky et al., 1987) and also by bezafibrate in patients with NIDDM (Seviour et al., 1988; Rovellini et al., 1992; Niort et al., 1992). Increased concentrations of HDL cholesterol are most obvious when levels are initially low in patients with hypertriglyceridaemia, treated with bezafibrate (Stewart et al., 1982), and response is delayed in comparison with changes in total serum cholesterol and triglyceride (Vessby et al., 1980). In the present study, longer treatment time may have been required to significantly elevate HDL cholesterol. There is uncertainty whether it is HDL_2 or HDL_3 that is increased in response to bezafibrate (Seviour et al., 1988). Lecithin-cholesterol acyl transferase activity in NIDDM patients does not appear to be affected by bezafibrate (Prager et al., 1982; Heller et al., 1983) and therefore bezafibrate-induced elevated HDL cholesterol is likely to be mediated by increased LPL activity and therefore elevated VLDL catabolism, and less cholesterol ester transfer between HDL and triglyceride-rich lipoproteins.

In the present study, fasting and postprandial levels of total and LDL cholesterol fell after bezafibrate treatment, although not significantly so. As with HDL cholesterol, differences in LDL cholesterol levels may have been more apparent with longer treatment time. This is consistent with the findings of Rovellini et al. (1992) and Niort et al. (1992), who reported that serum cholesterol was significantly reduced in hypertriglyceridaemic

patients with NIDDM following bezafibrate therapy. However, bezafibrate has been shown to have no effect on LDL cholesterol (Olsson et al. 1985; Vessby et al., 1982) and to increase LDL cholesterol in patients with hypertriglyceridaemia (Saku et al., 1989) the latter being mediated by more normal and rapid conversion of VLDL to LDL resulting in the restoration of cholesterol ester-rich LDL which was previously triglyceride-rich due to cholesterol ester transfer. In contrast, increased receptor mediated catabolism of LDL has been demonstrated *in vivo* after bezafibrate therapy (Series et al., 1989; Stewart et al., 1982) resulting in depressed levels of LDL cholesterol. This may be caused by increased receptor expression mediated by enhanced secretion of cholesterol into the bile, known to occur after fibrate therapy. Alternatively, changes in LDL composition may provoke more avid binding to the receptors. Studies have shown statins to be better at reducing LDL cholesterol levels and fibrates better at reducing triglyceride levels and increasing HDL levels (Crepaldi et al., 1990; Ojala et al., 1990). Fibrates have also been shown to be better than statins at reducing postprandial lipaemia (Simo et al., 1993); in hypertriglyceridaemic subjects gemfibrozil reduced chylomicron triglyceride and there was a close correlation between change in fasting triglyceride and change in postprandial response in subjects taking gemfibrozil but not in those taking lovastatin. Also LPL and HL activities were increased by gemfibrozil but not by lovastatin.

AUC for NEFA was significantly reduced following bezafibrate therapy, providing less substrate for VLDL synthesis and contributing to the reduction in VLDL. Similarly, previous studies have reported a reduction of NEFA concentration after bezafibrate therapy in patients with NIDDM (Alberti et al., 1990; Jones et al., 1990). A reduction in NEFA levels would suggest an increase in insulin sensitivity. Bezafibrate-induced reduction of serum triglyceride levels might improve insulin-binding to cell receptors (Bhathena et al., 1989) and the decrease of plasma NEFA, might mediate an increase in insulin sensitivity by increasing insulin-stimulated glucose uptake (Fraze et al., 1985) and by decreasing hepatic glucose production (Ferrannini et al., 1983). However there was no significant difference in insulin levels, glucose levels or insulin resistance following bezafibrate treatment. Insulin levels, and therefore values of insulin resistance and β-cell function, increased in both treatment groups and although this is clearly not an effect of bezafibrate, the reason for increased insulin levels is less clear. IAUC for glucose was significantly increased following bezafibrate treatment, but this change was small (Fig. 8) and perhaps not a good indication of the true effect of the drug on glucose concentrations. The effect on bezafibrate on glucose concentrations in NIDDM is not fully understood. Riccardi et al. (1989) demonstrated that bezafibrate had no effect on fasting glucose concentration, glucose

tolerance, insulin secretion or peripheral insulin sensitivity in hyperlipidaemic patients with and without diabetes. In contrast, previous studies in NIDDM have reported that bezafibrate lowers glucose levels (Seviour et al., 1988; Jones and Alberti, 1988; Mikhailidis et al.,1990; Rovellini et al., 1992). In some studies, fasting as well as postprandial glucose concentrations were reduced (Jones et al., 1990; Smud and Sermukslis, 1987), and dosage reductions in glycaemic control medication was necessary in some of the patients (Smud and Sermukslis, 1987).

To summarise, bezafibrate therapy resulted in reductions in fasting and postprandial triglyceride-rich lipoproteins and small dense LDL towards that of normolipidaemic subjects. Reductions were particularly apparent in large VLDL and postprandial chylomicrons. Bezafibrate therapy also decreased cholesterol ester content in VLDL subfractions, reduced triglyceride content in HDL and increased the proportion of small VLDL accompanying a decrease in the proportion of large VLDL. However, there was no effect on total, LDL and HDL cholesterol, HDL triglyceride, glucose and insulin levels. Bezafibrate clearly improves the VLDL and LDL subfraction profile in patients with NIDDM and therefore potentially reduces atherogenic risk.

CHAPTER 5

DYSLIPIDAEMIA IN SYSTEMIC LUPUS ERYTHEMATOSUS AND PSORIATIC ARTHRITIS

5.1. INTRODUCTION

Morbidity and mortality from CHD is common in subjects with systemic lupus erythematosus (Urowitz et al., 1976) and rheumatoid arthritis (Isomaki et al., 1975). In recent years, dyslipoproteinaemia has been noted in patients suffering from RA (Svenson et al., 1987a; Svenson et al., 1987b; Rantapaa-Dahlqvist et al., 1990; Lazarevic et al., 1992), PA (Lazarevic et al., 1992; Jones et al., 1993) and SLE (Ilowite et al.,1988; Leong et al.,1994). However, these studies have only investigated basic quantitative lipid and lipoprotein parameters. The aim of this study was to investigate quality as well as quantity of lipids and lipoproteins in patients with PA and SLE compared to healthy normolipidaemic controls, because of increasing evidence of the likely importance of these quality changes.

5.2. METHODS

5.2.1. Study population

1. 17 SLE patients and 17 PA were selected at random (by Dr.N.McHugh and Dr. S.Jones, Bath Hospital for Rheumatic Disease) from patients attending the Bath Hospital for Rheumatic Disease (Table 5.1 and 5.2).

2. 17 age- and gender-matched controls for each patient group were selected from the same geographical location (Table 5.1 and 5.2).

	SLE patients	Controls
NUMBER	17	17
AGE (yrs) ± SEM	42.3± 3.07	43.9± 2.93
GENDER	12 female, 5 male	12 female, 5 male
DISEASE ACTIVITY	13 inactive, 4 active	
THERAPY	3 untreated, others on corticosteroids, hydroxychloroquine, and other non-steroidal antiinflammatory drugs	

Table 5.1. Demographic data for SLE patients and controls.

	PA patients	Controls
NUMBER	17	17
AGE (yrs)± SEM	42.9± 3.89	42.5± 3.34
GENDER	10 female, 7 male	10 female, 7 male
DISEASE ACTIVITY	13 active, 4 inactive	
THERAPY	All patients on non-steroidal antiinflammatory drugs	

Table 5.2. Demographic data for PA patients and controls.

5.2.2. Measurements

Fasting blood samples were taken from all subjects for determination of apolipoprotein AI and B in serum and cholesterol and triglyceride in serum and serum lipoproteins (HDL, HDL_2 and HDL_3) (data supplied by C.Stirling and co-workers). Measurements for each subfraction included total cholesterol, triglyceride, phospholipid, free cholesterol, esterified cholesterol and total protein. The mass of each fraction was calculated as the sum of the latter five components. Composition of these fractions was expressed as a percentage of total mass. Fasting apo B concentrations were determined in VLDL-1, -2 and -3 and LDL-1 fractions only (see Methods 2.3.7). Cholesterol and triglyceride values for VLDL and LDL were calculated as the sum of these lipid components in VLDL and LDL subfractions respectively. Chylomicron measurements for patients and controls were on the whole very low, sometimes undetectable, and therefore chylomicron data has not been presented.

5.2.3. Statistics

Comparisons between patients and controls were made using an unpaired t-test. Data for triglycerides was log-transformed prior to analysis. Data are presented as mean ± SEM.

5.3. RESULTS

5.3.1. SLE patients and their controls

5.3.1.1. All SLE patients (males and females, n=17)

SLE patients had higher levels of serum and VLDL triglyceride, and lower levels of HDL cholesterol (p=0.054) and apo AI compared to controls, contributing to an atherogenic lipoprotein profile, although differences did not achieve significance (Table 5.3).

Although there were no quantitative differences between SLE patients and controls in VLDL and LDL subfractions (Table 5.4), some qualitative differences were seen. SLE patients had significantly less % cholesterol in the VLDL subfractions, which was reflected in trends to higher % triglyceride in these subfractions (Table 5.5). SLE patients also had significantly higher % LDL-3/ΣLDL mass ratio compared to controls (Table 5.6). Because serum triglycerides were slightly elevated in SLE patients and because of the known association between triglyceride and small, dense LDL (Austin 1986, 1988), subjects were matched for triglyceride levels. The difference in % LDL-3/ΣLDL mass ratio no longer reached significance (p=0.052) after matching for triglyceride levels (Table 5.7), however the mean values remained unchanged and the p value was only marginally greater than 0.05 suggesting that this lack of significance may be due to the smaller number of patients and not the matching of triglyceride levels. LDL/HDL cholesterol and HDL triglyceride/cholesterol ratios tended to be higher and HDL_2/HDL_3 cholesterol ratio tended to be lower in SLE patients compared to controls. The VLDL subfraction profile (% of VLDL subfractions in total VLDL mass) was similar in both subject groups as was the particle size within each subfraction (triglyceride/apo B ratio) (Table 5.6).

Table 5.3. Lipids, lipoproteins and apolipoproteins in 17 SLE patients and their controls.

	SLE patients	Controls
Cholesterol (mM)	5.54±0.30	5.46±0.31
Triglyceride (mM)	1.39±0.19	1.12±0.11
VLDL cholesterol (mM)	0.34±0.05	0.34±0.06
VLDL triglyceride (mM)	0.66±0.13	0.44±0.08
LDL cholesterol (mM)	2.71±0.22	2.52±0.15
LDL triglyceride (mM)	0.22±0.03	0.22±0.02
HDL cholesterol (mM)	1.26±0.10	1.61±0.14
HDL_2 cholesterol (mM)	0.59±0.10	0.83±0.13
HDL_3 cholesterol (mM)	0.68±0.04	0.78±0.07
HDL triglyceride (mM)	0.22±0.02	0.23±0.01
Apo B (mg/dl)	75.6±6.48	70.5±5.38
Apo AI (mg/dl)	154±9.48	170±8.16

All values are mean ± SEM.

Table 5.4. Concentrations of lipid and protein components of VLDL and LDL subfractions in 17 SLE patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr
CH	1.94± 0.68	1.86± 0.62	3.10± 0.70	2.68± 0.49	8.03± 0.92	8.67± 1.47	14.1± 1.81	16.8± 2.38	63.5± 6.02	61.8± 3.94	27.3± 3.26	18.9± 3.33
TG	17.5± 5.87	10.5± 2.28	18.6± 4.43	12.3± 2.81	22.72 ±2.93	16.5± 2.52	8.47± 1.31	8.10± 0.92	9.07± 1.45	8.56± 0.80	2.31± 0.40	2.53± 0.43
PL	3.87± 1.38	2.14± 0.64	4.98± 1.21	3.37± 0.65	9.41± 1.07	8.25± 1.19	10.6± 1.44	11.2± 1.79	38.4± 3.04	38.2± 2.49	16.5± 1.85	11.3± 2.44
EC	1.33± 0.40	1.46± 0.46	2.35± 0.54	2.21± 0.39	7.17± 0.91	8.43± 1.80	15.03 ±2.11	17.7± 2.82	75.1± 7.97	71.2± 5.67	33.4± 4.52	22.0± 3.93
FC	1.15± 0.36	0.99± 0.35	1.70± 0.40	1.37± 0.28	3.76± 0.40	3.65± 0.51	5.15± 0.75	6.27± 0.92	18.8± 1.68	19.4± 1.23	7.40± 0.71	5.78± 1.06
Prot	2.85± 1.38	1.80± 0.61	3.76± 1.08	2.50± 0.50	8.19± 0.91	6.33± 0.80	12.1± 1.27	10.2± 1.07	46.4± 3.14	40.4± 2.56	24.4± 2.69	16.5± 3.53
Apo B	1.88± 0.98	1.25± 0.51	2.29± 0.71	1.37± 0.26	6.09± 0.66	4.53± 0.62	11.0± 1.18	8.91± 1.00				
Mass	26.7± 9.31	16.8± 3.83	31.4± 7.59	21.8± 4.18	51.3± 5.47	43.2± 6.07	51.4± 6.25	53.5± 7.01	187.9 ±15.5	177.8 ±10.4	84.0± 9.57	58.1± 11.2

All concentrations are mean ± SEM (mg/dl).

Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

Table 5.5. Composition of VLDL and LDL subfractions in 17 SLE patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr
CH	7.76± 0.28	9.84± 0.97 a	9.76± 0.41	12.7± 1.09 a	15.7± 0.73	19.5± 0.95 b	26.9± 0.80	30.9± 0.93	33.3± 0.75	34.8± 0.73	32.2± 0.60	33.1± 0.72
TG	66.4± 1.09	63.0± 3.40	59.7± 0.89	53.7± 3.90	43.7± 2.49	37.8± 2.48	16.6± 1.49	15.7± 0.88	4.72± 0.55	4.85± 0.31	2.75± 0.40	4.56± 0.28 b
PL	14.3± 0.82	14.1± 2.04	15.3± 0.62	16.7± 1.72	18.6± 0.93	19.7± 0.96	20.2± 0.68	20.7± 0.87	20.7± 0.72	21.4± 0.60	19.8± 0.67	18.7± 0.83
EC	5.97± 0.51	8.43± 0.88 a	7.43± 0.48	10.9± 1.11 b	13.9± 0.83	17.8± 1.70 a	28.0± 2.04	31.6± 2.30	39.1± 1.76	39.9± 1.44	38.6± 1.77	38.3± 1.74
FC	4.20± 0.33	5.13± 0.67	5.34± 0.34	6.19± 0.81	7.45± 0.32	8.96± 0.64 a	10.2± 0.87	12.1± 1.19	10.1± 1.47	11.1± 0.60	9.25± 0.55	10.3± 0.84
Prot	9.18± 0.82	9.61± 0.89	12.2± 0.69	12.5± 1.21	16.4± 0.75	15.8± 0.84	24.9± 0.95	19.8± 0.52 c	25.4± 0.87	22.8± 0.70 a	29.6± 0.96	28.1± 0.52

All values are mean % of total mass ± SEM.

Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Table 5.6. Qualitative parameters in 17 SLE patients compared to controls.

	SLE patients	Controls	
%VLDL-1/ ∑VLDL	19.6±2.39	18.4±1.93	
%VLDL-2/ ∑VLDL	26.3±1.73	26.2±1.52	
%VLDL-3/ ∑VLDL	54.1±3.42	55.3±2.25	
%LDL-3/∑LDL	26.5±2.38	18.9±1.93	a
LDL chol/ HDL chol	2.37±0.28	1.74±0.19	
HDL_2/ HDL_3 chol	0.96±0.19	1.21±0.20	
HDL trig/chol	0.19±0.02	0.16±0.01	
VLDL-1 trig/apo B	13.6±1.63	12.8±2.24	
VLDL-2 trig/apo B	10.2±2.28	9.64±1.53	
VLDL-3 trig/apo B	3.85±0.46	3.66±0.39	

All values are mean ± SEM.
chol: cholesterol; trig: triglyceride.
a: $p<0.05$

Table 5.7. Lipid and lipoprotein parameters in 13 SLE patients compared to controls, matched for triglyceride.

	SLE patients(n=13)	Controls (n=13)
Triglyceride (mM)	1.10±0.08	1.12±0.13
%LDL-3/∑LDL	26.2±2.36	18.8±2.70 (p=0.052)

All values are mean ± SEM.
SLE patients and controls were matched for triglyceride by excluding 4 SLE patients with the highest triglyceride levels and their age-matched controls.

5.3.1.2. Female SLE patients (n=12)

For female subjects, there were similar trends in lipid and lipoprotein parameters as for all subjects. SLE females had elevated serum and VLDL triglyceride, and lower HDL cholesterol and apo AI levels compared to controls (Table 5.8).

The concentration of LDL-3 and most of its lipid and protein components were significantly elevated in SLE females (Table 5.9). Percentage esterified cholesterol in VLDL-1, -2, -3 and % cholesterol in LDL-1 were significantly lower in SLE females, which was reflected in higher (only significant for VLDL-3) % triglyceride in these subfractions (Table 5.10).

SLE females had significantly higher % LDL-3/ΣLDL mass ratio (Table 5.11), which persisted when subjects were matched for serum triglyceride (Table 5.12). Mean HDL_2/HDL_3 cholesterol ratio was substantially reduced and LDL/HDL cholesterol and HDL triglyceride/cholesterol ratios tended to be raised in SLE females compared to controls (Table 5.12). There were no differences in VLDL subfraction profile or particle size (Table 5.12). These changes in female patients were quantitatively and qualitatively quite similar to those for the whole group, although there were some changes in significance levels. The LDL-3 changes were overall more marked in the females compared to the whole group.

Table 5.8. Lipids, lipoproteins and apolipoproteins in 12 female SLE patients and their controls

	SLE patients	Controls
Cholesterol (mM)	5.23±0.36	5.40±0.35
Triglyceride (mM)	1.19±0.11	0.99±0.12
VLDL cholesterol (mM)	0.28±0.05	0.32±0.08
VLDL triglyceride (mM)	0.53±0.10	0.36±0.07
LDL cholesterol (mM)	2.46±0.24	2.45±0.16
LDL triglyceride (mM)	0.23±0.04	0.21±0.02
HDL cholesterol (mM)	1.31±0.14	1.68±0.17
HDL_2 cholesterol (mM)	0.62±0.14	0.99±0.15
HDL_3 cholesterol (mM)	0.69±0.05	0.69±0.06
HDL triglyceride (mM)	0.22±0.02	0.24±0.02
Apo B (mg/dl)	67.8±5.70	65.0±4.64
Apo AI (mg/dl)	161.3±11.8	175.1±10.9

All values are mean ± SEM.

Table 5.9. Concentrations of lipid and protein components of VLDL and LDL subfractions in 12 female SLE patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr
CH	1.42± 0.42	1.67± 0.82	2.37± 0.56	2.15± 0.50	7.20± 1.14	8.60± 1.99	12.6± 2.17	17.7± 3.17	61.4± 7.33	63.6± 5.16	21.1± 2.06	13.5± 0.91 b
TG	11.8± 3.39	7.81± 1.85	13.3± 2.88	9.66± 2.10	21.5± 3.60	14.7± 2.44	8.65± 1.62	8.19± 1.26	9.65± 1.78	8.74± 1.09	2.05± 0.44	1.97± 0.24
PL	2.34± 0.72	1.86± 0.77	3.58± 0.82	2.42± 0.43	8.08± 1.22	7.65± 1.51	9.40± 1.70	11.6± 2.48	37.5± 3.99	38.4± 3.29	12.8± 1.04	7.37± 0.75 c
EC	1.01± 0.35	1.41± 0.62	1.79± 0.48	1.96± 0.45	6.40± 1.15	8.80± 2.47	13.3± 2.57	18.9± 3.69	71.5± 9.58	73.9± 7.39	25.6± 3.34	15.8± 1.36 a
FC	0.81± 0.23	0.84± 0.45	1.30± 0.29	0.98± 0.26	3.39± 0.49	3.37± 0.66	4.70± 0.93	6.43± 1.27	18.8± 2.22	19.6± 1.61	5.93± 0.36	4.04± 0.44 b
Prot	1.40± 0.44	1.60± 0.77	2.49± 0.48	1.79± 0.28	7.21± 0.94	5.92± 0.97	11.2± 1.52	10.2± 1.36	45.1± 3.86	39.5± 3.19	19.1± 1.12	11.5± 0.81 c
Apo B	0.81± 0.22	1.25± 0.67	1.05± 0.19	0.97± 0.19	5.43± 0.66	4.39± 0.79	10.1± 1.41	8.90± 1.31				
Mass	17.4± 5.07	13.5± 4.18	22.5± 4.85	16.8± 3.31	46.5± 6.41	40.4± 7.44	47.2± 7.62	55.3± 9.39	183± 18.9	180± 13.7	65.4± 5.58	40.7± 2.80 c

All concentrations are mean ±SEM (mg/dl).

Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Table 5.10. Composition of VLDL and LDL subfractions in 12 female SLE patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr
CH	7.99± 0.33	9.92± 1.31	9.97± 0.57	12.4± 1.24	15.3± 0.94	20.1± 1.19 b	26.0± 1.04	31.6± 1.16 b	33.1± 1.02	35.4± 0.89	32.0± 0.75	33.2± 0.97
TG	67.4± 1.23	61.9± 4.14	59.3± 1.18	56.2± 3.16	45.6± 2.87	37.3± 2.66 a	18.5± 1.49	15.3± 1.09	5.27± 0.64	4.89± 0.43	2.95± 0.49	4.78± 0.36
PL	13.3± 0.50	14.4± 2.58	15.2± 0.85	15.1± 1.33	17.5± 0.89	19.3± 1.02	19.4± 0.46	20.4± 1.12	20.6± 0.89	21.2± 0.77	19.8± 0.88	18.0± 1.12
EC	6.40± 0.67	9.23± 1.15 a	7.43± 0.62	11.7± 1.37 b	13.5± 1.13	18.9± 2.24 a	26.5± 2.73	33.1± 2.83	38.4± 2.40	40.8± 1.07	37.9± 2.33	38.8± 2.32
FC	4.18± 0.45	4.84± 0.90	5.55± 0.42	5.39± 0.93	7.33± 0.38	8.81± 0.87	10.3± 1.19	11.9± 1.56	10.2± 0.63	11.0± 0.79	9.48± 0.75	10.1± 1.08
Prot	8.75± 1.04	9.99± 0.96	12.4± 0.90	11.6± 0.84	16.1± 0.87	15.7± 0.80	25.3± 1.19	19.3± 0.67 c	25.5± 1.20	21.9± 0.79 a	29.9± 1.19	28.3± 0.54

All values are mean % of total mass ± SEM.

Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Table 5.11. Qualitative parameters in 12 female SLE patients and their controls.

	SLE patients	Controls
%VLDL-1/ ∑VLDL	17.9±2.69	17.3±2.13
%VLDL-2/ ∑VLDL	24.5±1.94	25.1±1.91
%VLDL-3/ ∑VLDL	57.5±3.70	57.6±2.22
%LDL-3/∑LDL	23.6±2.50	15.0±0.92 b
LDL chol/ HDL chol	2.14±0.33	1.58±0.15
HDL_2/ HDL_3 chol	0.99±0.25	1.53±0.22
HDL trig/chol	0.19±0.03	0.16±0.02
VLDL-1 trig/apoB	14.6±1.93	11.2±2.29
VLDL-2 trig/apoB	10.73±3.19	9.64±1.54
VLDL-3 trig/apoB	4.03±0.59	3.34±0.34

All values are mean ± SEM.
chol: cholesterol; trig: triglyceride.
b: $p<0.01$

Table 5.12. Lipid and lipoprotein parameters in 9 female SLE patients compared to controls, matched for triglyceride.

	SLE Patients(n=9)	Controls (n=9)
Triglyceride (mM)	1.03±0.09	1.08±0.15
%LDL-3/∑LDL	23.9±1.98	15.1±1.23 b

SLE patients and controls were matched for triglyceride by excluding 3 SLE patients with the highest triglyceride levels and their age-matched controls.
All values are mean ± SEM.
b: $p<0.01$

5.3.1.3. Male SLE patients (n=5)

Male SLE patients tended to have higher mean levels of serum and VLDL triglyceride, serum and LDL cholesterol and lower mean levels of HDL cholesterol and apo AI (p=0.06) compared to controls although differences did not achieve significance (Table 5.13). HDL_3 cholesterol levels were significantly reduced in SLE males (Table 5.13).

There were no significant quantitative (Table 5.14), nor qualitative (Table 5.15) differences in VLDL and LDL subfractions between SLE males and controls, although mean % LDL-3/ΣLDL mass ratio again tended to be higher in SLE males (Table 5.16). In addition, LDL/HDL cholesterol and HDL triglyceride/cholesterol ratios were non-significantly higher in these patients (Table 5.16).

Quantitative and qualitative differences are not dissimilar to the female group, although differences in % LDL-3/ΣLDL mass ratio were less marked in the male group. Larger patient numbers are needed to confirm the aforementioned trends, since statistical analyses are not reliable with small patient numbers.

Table 5.13. Lipids, lipoproteins and apolipoproteins in SLE patients and controls

	SLE patients	Controls	
Cholesterol (mM)	6.26±0.38	5.59±0.70	
Triglyceride (mM)	1.86±0.57	1.40±0.24	
VLDL cholesterol (mM)	0.47±0.12	0.39±0.09	
VLDL triglyceride (mM)	1.00±0.37	0.64±0.22	
LDL cholesterol (mM)	3.32±0.38	2.69±0.35	
LDL triglyceride (mM)	0.21±0.05	0.22±0.04	
HDL cholesterol (mM)	1.17±0.07	1.44±0.23	
HDL_2 cholesterol (mM)	0.53±0.12	0.45±0.11	
HDL_3 cholesterol (mM)	0.64±0.06	0.99±0.13	a
HDL triglyceride (mM)	0.21±0.04	0.20±0.01	
Apo B (mg/dl)	99.0±15.4	83.8±13.7	
Apo AI (mg/dl)	132.5±6.99	159.0±8.89	

All values are mean ± SEM.
a: $p<0.05$

Table 5.14. Concentrations of lipid and protein components of VLDL and LDL subfractions in 5 male SLE patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr
CH	3.20± 1.71	2.31± 0.80	4.87± 1.89	3.97± 1.01	10.0± 1.21	8.84± 1.76	17.7± 3.01	14.7± 3.04	68.8± 11.3	57.6± 5.37	42.1± 6.29	31.8± 9.24
TG	30.9± 18.1	16.8± 5.79	31.4± 12.4	18.7± 8.01	25.8± 5.23	20.9± 6.33	8.06± 2.42	7.88± 1.01	7.69± 2.68	8.12± 0.94	2.97± 0.86	3.87± 1.23
PL	7.53± 4.20	2.82± 1.21	8.35± 3.40	5.65± 1.65	12.6± 1.4	9.67± 1.86	13.5± 2.48	10.4± 1.70	40.7± 4.29	37.6± 3.61	25.3± 3.47	20.6± 6.82
EC	2.09± 1.06	1.58± 0.52	3.70± 1.32	2.80± 0.79	9.01± 1.13	7.57± 1.83	19.3± 3.24	14.9± 3.92	83.7± 15.2	64.9± 7.88	52.3± 8.86	36.7± 11.0
FC	1.95± 1.08	1.36± 0.52	2.67± 1.11	2.30± 0.54	4.65± 0.56	4.34± 0.69	6.22± 1.19	5.86± 0.93	18.9± 2.43	19.0± 1.84	10.9± 1.24	9.97± 2.78
Prot	6.34± 4.50	2.27± 1.02	6.81± 3.32	4.21± 1.35	10.6± 1.83	7.31± 1.49	14.3± 2.28	10.3± 1.81	49.7± 5.67	42.5± 4.51	37.1± 5.67	28.7± 10.6
Apo B	4.45± 3.21	1.26± 0.46	4.41± 2.21	2.10± 0.66	7.70± 1.46	4.87± 0.99	13.1± 2.08	8.94± 1.54				
Mass	48.9± 28.9	24.9± 7.84	52.9± 21.6	33.6± 10.7	62.6± 9.54	49.8± 10.9	61.3± 10.7	49.33 ±8.93	201± 29.6	172± 15.3	128± 18.3	99.8± 32.2

All concentrations are mean ±SEM (mg/dl).

Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

Table 5.15. Composition of VLDL and LDL subfractions in 5 male SLE patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr	SLE	Contr
CH	7.19± 0.47	9.68± 1.22	9.25± 0.36	13.5± 2.41	16.5± 1.07	18.2± 1.50	29.0± 0.19	29.2± 1.34	33.9± 0.79	33.5± 1.18	32.7± 1.05	32.9± 0.87
TG	63.9± 2.00	65.6± 6.44	60.6± 1.11	47.5± 11.3	39.1± 4.71	39.0± 6.05	12.1± 2.89	16.8± 1.52	3.39± 0.93	4.75± 0.38	2.28± 0.54	4.03± 0.32
PL	16.7± 2.32	13.4± 3.53	15.6± 0.62	20.5± 4.85	21.2± 2.05	20.8± 2.30	22.2± 1.87	21.6± 1.29	20.9± 1.37	21.8± 0.91	19.7± 0.96	20.4± 0.40
EC	4.95± 0.45	6.55± 0.64	7.42± 0.79	9.07± 1.78	14.8± 0.77	15.0± 1.87	31.7± 1.66	28.2± 3.82	40.8± 1.74	37.7± 2.68	40.4± 2.34	37.2± 2.30
FC	4.25± 0.26	5.78± 0.85	4.83± 0.57	8.11± 1.41	7.74± 0.65	9.30± 0.74	10.1± 0.88	12.4± 1.77	9.62± 0.62	11.1± 0.93	8.71± 0.54	10.8± 1.32
Prot	10.2± 1.27	8.66± 2.10	11.6± 1.00	14.8± 3.67	17.2± 1.58	15.9± 2.32	23.9± 1.59	21.0± 0.44	25.2± 0.96	24.6± 1.15	28.9± 1.71	27.6± 1.29

All values are mean % of total mass ± SEM.

Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

Table 5.16. Qualitative parameters in 5 male SLE patients and their controls.

	SLE patients	Controls
%VLDL-1/ ∑VLDL	23.7±4.86	21.3±4.21
%VLDL-2/ ∑VLDL	30.5±3.10	28.9±2.22
%VLDL-3/ ∑VLDL	45.8±6.65	49.8±5.09
%LDL-3/∑LDL	33.5±4.27	28.2±4.88
LDL chol/ HDL chol	2.94±0.51	2.14±0.51
HDL_2/ HDL_3 chol	0.90±0.25	0.44±0.06
HDL trig/chol	0.18±0.03	0.15±0.02
VLDL-1 trig/apoB	11.5±3.12	17.9±5.63
VLDL-2 trig/apoB	9.08±1.77	9.63±3.88
VLDL-3 trig/apoB	3.43±0.71	4.23±1.12

All values are mean ± SEM.
chol: cholesterol; trig: triglyceride.

5.3.2. Patients with PA and their controls

5.3.2.1. All PA patients (males and females, n=17)

Basic lipid and lipoprotein parameters are shown in Table 5.17. PA patients had significantly reduced levels of HDL, HDL_2 and HDL_3 cholesterol which was accompanied by significantly lower serum apo AI concentration.

There were no quantitative differences in VLDL and LDL subfractions between PA patients and controls (Table 5.18). However, PA patients had significantly reduced % cholesterol in LDL-1, reflected in a non-significant increase in % triglyceride (Table 5.19). Percentage triglyceride was increased in LDL-2 in PA patients compared to controls. These qualitative differences in LDL subfractions are isolated and only small and weakly significant and may therefore be chance differences. However, reduced % cholesterol in LDL-1 with a non-significant increase in % triglyceride also occurred in female patients (section 5.3.2.2) and increased % triglyceride in LDL-2 also occurred in male patients (section 5.3.2.3), suggesting that these differences are genuine.

LDL/HDL cholesterol and HDL triglyceride/cholesterol ratios were significantly raised in PA patients (Table 5.20). Other qualitative differences, although not significant, include reduced HDL_2/HDL_3 cholesterol ratio and increased %LDL-3/ΣLDL mass ratio ($p=0.062$) in PA patients compared to controls (Table 5.20). VLDL subfraction profile and particle size was similar in both subject groups (Table 5.20).

Table 5.17. Lipids, lipoproteins and apolipoproteins in 17 PA patients and their controls

	PA patients	Controls	
Cholesterol (mM)	4.83±0.24	5.20±0.31	
Triglyceride (mM)	1.21±0.16	1.12±0.09	
VLDL cholesterol (mM)	0.36±0.08	0.33±0.05	
VLDL triglyceride (mM)	0.56±0.11	0.46±0.07	
LDL cholesterol (mM)	2.36±0.13	2.55±0.21	
LDL triglyceride (mM)	0.26±0.02	0.21±0.02	
HDL cholesterol (mM)	1.01±0.06	1.52±0.11	c
HDL_2 cholesterol (mM)	0.49±0.05	0.69±0.07	a
HDL_3 cholesterol (mM)	0.53±0.05	0.83±0.09	b
HDL triglyceride (mM)	0.24±0.02	0.22±0.02	
Apo B (mg/dl)	71.3±6.74	66.6±4.92	
Apo AI (mg/dl)	122±13.7	161±5.41	a

All values are mean ± SEM.

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Table 5.18. Concentrations of lipid and protein components of VLDL and LDL subfractions in 17 PA patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr
CH	1.62± 0.46	1.38± 0.30	2.97± 0.83	2.64± 0.44	9.51± 2.07	8.85± 1.30	13.3± 1.45	15.8± 1.67	54.2± 2.75	62.8± 5.22	24.0± 3.10	20.0± 3.64
TG	12.9± 3.46	10.2± 1.74	15.2± 3.54	12.7± 2.42	21.5± 3.85	17.7± 2.40	9.18± 0.90	7.76± 0.74	10.2± 1.04	8.50± 0.87	3.73± 0.54	2.68± 0.44
PL	2.42± 0.70	1.96± 0.41	3.95± 1.01	3.75± 0.66	9.95± 1.85	8.76± 1.13	9.72± 0.87	10.4± 1.07	34.1± 2.57	38.1± 3.15	14.5± 1.71	11.9± 2.58
EC	1.34± 0.26	1.08± 0.21	2.41± 0.52	2.17± 0.36	8.70± 1.73	8.85± 1.68	15.3± 1.66	17.5± 2.22	62.0± 3.35	73.9± 6.91	28.8± 3.80	24.0± 4.39
FC	0.88± 0.31	0.74± 0.18	1.54± 0.52	1.35± 0.25	4.33± 1.08	3.58± 0.40	4.21± 0.52	5.44± 0.58	17.3± 0.93	18.8± 1.56	6.83± 1.02	5.65± 1.09
Prot	1.68± 0.45	1.50± 0.33	2.97± 0.72	3.08± 0.57	7.76± 1.44	7.05± 0.85	9.26± 0.71	10.2± 0.93	39.0± 1.72	41.0± 3.87	20.1± 2.79	17.1± 3.74
Apo B	1.04± 0.28	0.82± 0.15	1.68± 0.42	1.83± 0.37	5.62± 1.09	5.07± 0.68	8.27± 0.69	8.84± 0.85				
Mass	19.1± 5.13	15.5± 2.73	26.1± 6.28	23.1± 3.89	52.3± 9.77	45.9± 6.00	47.7± 4.16	51.2± 4.90	163± 8.48	180± 14.6	73.9± 9.48	61.4± 12.0

All concentrations are mean ± SEM (mg/dl).
Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol;
FC: free cholesterol; Prot: protein.

Table 5.19. Composition of VLDL and LDL subfractions in 17 PA patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr
CH	8.90± 0.74	9.07± 0.86	10.9± 0.65	12.0± 1.05	17.7± 0.56	19.1± 0.83	27.5± 0.91	30.6± 0.92 a	33.4± 0.44	34.9± 0.67	32.3± 0.70	32.8± 0.91
TG	65.1± 1.45	65.5± 2.24	58.1± 1.11	53.4± 3.51	40.7± 1.08	38.1± 1.90	19.6± 1.44	15.5± 1.06	6.24± 0.52	4.85± 0.40 a	5.02± 0.28	5.40± 1.02
PL	11.6± 0.49	12.8± 1.13	15.0± 0.32	16.9± 1.53	18.9± 0.31	19.5± 0.76	20.3± 0.45	20.3± 0.71	20.7± 0.63	21.2± 0.60	20.4± 1.21	18.5± 0.82
EC	9.13± 1.23	7.47± 0.83	10.5± 0.96	10.3± 1.05	17.4± 1.04	18.1± 1.58	31.7± 1.43	33.1± 1.97	38.1± 0.56	40.8± 1.332	38.5± 1.41	39.5± 1.69
FC	3.68± 0.59	4.91± 0.54	4.67± 0.60	5.86± 0.80	7.40± 0.51	8.36± 0.60	8.68± 0.42	10.9± 1.10	10.7± 0.37	10.6± 0.57	9.37± 0.93	9.30± 0.79
Prot	10.8± 0.92	9.63± 0.57	11.7± 0.38	13.5± 1.35	15.5± 0.55	16.0± 0.63	19.7± 0.37	20.1± 0.43	24.2± 0.51	22.5± 0.84	26.7± 1.06	27.2± 0.74

All values are mean % of total mass ± SEM.

Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$

Table 5.20. Qualitative parameters in 17 PA patients and their controls.

	PA patients	Controls	
%VLDL-1/ ∑VLDL	17.9±3.01	17.4±1.45	
%VLDL-2/ ∑VLDL	25.2±1.23	26.6±1.34	
%VLDL-3/ ∑VLDL	56.9±2.85	56.1±1.93	
%LDL-3/∑LDL	25.3±2.17	19.5±2.12	
LDL chol/ HDL chol	2.48±0.20	1.88±0.22	a
HDL_2/ HDL_3 chol	1.82±0.94	0.98±0.14	
HDL trig/chol	0.25±0.03	0.16±0.02	b
VLDL-1 trig/apoB	16.6±3.54	13.1±1.57	
VLDL-2 trig/apoB	9.91±1.05	8.47±1.26	
VLDL-3 trig/apoB	3.73±0.26	3.49±0.26	

All values are mean ± SEM.
chol: cholesterol; trig: triglyceride.
a: $p<0.05$

5.3.2.2. Female PA patients (n=10)

Quantitative and qualitative differences in females were not dissimilar to the whole group. HDL cholesterol was significantly reduced in female PA patients compared to controls, which was reflected in non-significant reductions in HDL_2, HDL_3 and apo AI (Table 5.21).

LDL-3 mass and cholesterol levels were significantly raised in female PA patients (Table 5.22). Qualitative changes included significantly reduced % cholesterol in LDL-1 in PA patients, reflected by elevated % triglyceride in the same subfraction (Table 5.23) as well as significantly elevated %LDL-3/ΣLDL mass ratio in PA patients (Table 5.24). In addition, LDL/HDL cholesterol and HDL triglyceride/cholesterol were substantially raised in SLE patients, whereas VLDL subfraction profile and particle size was similar in both subject groups.

Table 5.21. Lipids, lipoproteins and apolipoproteins in 10 female PA patients their controls

	PA patients	Controls	
Cholesterol (mM)	4.72±0.25	4.76±0.27	
Triglyceride (mM)	0.95±0.11	0.97±0.09	
VLDL cholesterol (mM)	0.22±0.04	0.29±0.06	
VLDL triglyceride (mM)	0.36±0.08	0.41±0.07	
LDL cholesterol (mM)	2.27±0.12	2.35±0.21	
LDL triglyceride (mM)	0.26±0.03	0.22±0.03	
HDL cholesterol (mM)	1.10±0.09	1.45±0.10	a
HDL_2 cholesterol (mM)	0.56±0.06	0.71±0.08	
HDL_3 cholesterol (mM)	0.54±0.08	0.74±0.10	
HDL triglyceride (mM)	0.25±0.03	0.25±0.02	
Apo B (mg/dl)	65.6±6.71	62.1±4.66	
Apo AI (mg/dl)	140±13.2	157±4.83	

All values are mean ± SEM.
a: $p<0.05$

Table 5.22. Concentrations of lipid and protein components of VLDL and LDL subfractions in 10 female PA patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr
CH	0.85± 0.28	0.94± 0.29	1.44± 0.28	2.08± 0.47	6.33± 1.25	8.38± 1.82	11.9± 1.41	16.0± 2.35	57.0± 2.70	61.4± 6.56	19.0± 2.01	13.6± 0.73 a
TG	7.97± 2.66	7.68± 1.55	8.79± 1.89	11.4± 2.29	15.5± 3.82	17.2± 2.54	8.86± 1.23	8.15± 1.04	10.7± 1.64	9.02± 1.29	3.06± 0.37	2.17± 0.29
PL	1.46± 0.49	1.10± 0.25	2.26± 0.49	2.48± 0.39	7.28± 1.64	7.55± 1.30	9.09± 0.94	9.78± 1.47	34.3± 2.77	36.6± 4.01	11.8± 1.10	7.12± 0.78 b
EC	0.76± 0.18	0.82± 0.24	1.47± 0.23	1.89± 0.45	6.15± 1.04	8.89± 2.58	13.8± 1.71	17.6± 3.31	66.1± 3.73	72.1± 9.20	22.7± 3.00	16.4± 1.34
FC	0.40± 0.19	0.45± 0.15	0.57± 0.17	0.96± 0.25	2.67± 0.66	3.09± 0.39	3.71± 0.48	5.52± 0.86	17.7± 0.67	18.4± 2.05	5.47± 0.83	3.87± 0.42
Prot	0.93± 0.17	0.94± 0.17	1.62± 0.26	1.90± 0.31	5.59± 0.99	6.04± 0.86	8.84± 0.85	9.52± 1.11	41.4± 2.25	37.7± 4.09	14.9± 1.73	10.9± 0.83
Apo B	0.59± 0.14	0.64± 0.13	0.93± 0.17	1.12± 0.21	4.21± 0.64	4.36± 0.65	7.91± 0.84	8.28± 1.06				
Mass	11.5± 3.54	11.0± 2.24	14.7± 2.96	18.6± 3.59	37.2± 7.99	42.7± 7.27	44.3± 4.54	50.5± 6.64	170± 9.35	174± 17.4	57.9± 5.73	40.5± 2.48 a

All concentrations are mean ± SEM (mg/dl).
Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol;
FC: free cholesterol; Prot: protein.
a: $p<0.05$; b $p<0.01$

Table 5.23. Composition of VLDL and LDL subfractions in 10 female PA patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr
CH	8.49± 1.11	8.14± 1.05	10.4± 1.01	10.8± 0.86	17.5± 0.75	18.7± 1.04	26.9± 1.30	31.3± 1.45 a	33.7± 0.67	35.3± 1.02	32.5± 1.12	34.0± 10.6
TG	64.2± 2.36	69.6± 1.69	58.1± 1.81	60.7± 1.70	39.9± 1.51	41.3± 1.64	19.8± 2.06	16.6± 1.63	6.20± 0.85	5.33± 0.57	5.31± 0.43	5.24± 0.52
PL	11.5± 0.67	10.5± 1.10	15.1± 0.30	13.8± 0.65	19.1± 0.51	17.8± 0.59	20.5± 0.64	19.3± 1.07	20.0± 0.76	21.0± 0.93	21.2± 2.00	17.4± 1.25
EC	9.89± 2.03	7.26± 1.19	11.3± 1.57	10.3± 1.44	18.0± 1.49	18.3± 2.52	31.1± 2.07	33.5± 3.30	38.9± 0.74	41.4± 2.06	38.3± 2.27	40.6± 2.62
FC	2.89± 0.68	4.24± 0.66	3.69± 0.69	4.61± 0.88	6.78± 0.56	7.81± 0.88	8.46± 0.56	11.4± 1.88	10.5± 0.43	10.7± 0.95	9.74± 1.55	9.82± 1.29
Prot	11.8± 1.49	8.82± 0.56	11.8± 0.55	10.5± 0.50	16.2± 0.85	14.8± 0.62	20.2± 0.52	19.2± 0.48	24.2± 0.64	21.5± 1.15 a	25.5± 1.70	26.9± 1.06

All values are mean % of total mass ± SEM.

Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$

Table 5.24. Qualitative parameters in 10 female PA patients and their controls.

	PA patients	Controls	
%VLDL-1/ ∑VLDL	17.7±4.52	14.8±1.28	
%VLDL-2/ ∑VLDL	23.7±1.65	26.1±1.96	
%VLDL-3/ ∑VLDL	58.6±3.42	59.1±1.99	
%LDL-3/∑LDL	21.2±1.51	15.7±0.94	b
LDL chol/ HDL chol	2.20±0.23	1.68±0.16	
HDL_2/ HDL_3 chol	2.56±1.58	1.15±0.22	
HDL trig/chol	0.24±0.04	0.18±0.02	
VLDL-1 trig/apoB	18.0±6.10	13.3±2.32	
VLDL-2 trig/apoB	10.1±1.67	11.0±1.61	
VLDL-3 trig/apoB	3.32±0.34	3.95±0.23	

All values are mean ± SEM.
chol: cholesterol; trig: triglyceride.
b: $p<0.01$

5.3.2.3. Male PA patients (n=7)

Male psoriatic patients had significantly reduced levels of HDL and HDL_3 cholesterol and apo AI compared to controls (Table 5.25). In addition serum and VLDL triglyceride tended to be higher in PA patients.

Although there were no quantitative differences in VLDL and LDL subfractions between male PA patients and controls, there were some qualitative differences (Table 5.26). Percentage triglyceride in LDL-2 was significantly increased (Table 5.27), and triglyceride/apoB ratio in VLDL-2 and -3 was higher in male PA patients than in controls (Table 5.28), suggesting that these particles were larger and more triglyceride-rich. LDL/HDL cholesterol and HDL triglyceride/cholesterol ratios were raised in SLE patients, the latter reaching significance. Percentage LDL-3/ΣLDL mass ratio was substantially higher in male PA patients, although the difference did not reach statistical significance.

Differences in male PA patients were similar to females in that they had reduced levels of HDL cholesterol compared to controls. However, males patients tended to have higher serum and VLDL triglyceride than controls which may have contributed to the higher % LDL-3/ΣLDL mass ratio in these patients; whereas females tended to have lower serum and VLDL triglyceride than controls and still had higher % LDL-3/ΣLDL mass ratio.

Table 5.25. Lipids, lipoproteins and apolipoproteins in 7 male PA patients and their controls

	PA patients	Controls	
Cholesterol (mM)	5.00±0.47	5.83±0.60	
Triglyceride (mM)	1.58±0.33	1.33±0.17	
VLDL cholesterol (mM)	0.57±0.17	0.39±0.08	
VLDL triglyceride (mM)	0.84±0.22	0.53±0.14	
LDL cholesterol (mM)	2.50±0.26	2.83±0.40	
LDL triglyceride (mM)	0.27±0.03	0.21±0.03	
HDL cholesterol (mM)	0.89±0.08	1.63±0.25	a
HDL_2 cholesterol (mM)	0.38±0.07	0.67±0.12	
HDL_3 cholesterol (mM)	0.50±0.03	0.96±0.14	a
HDL triglyceride (mM)	0.22±0.02	0.17±0.02	
Apo B (mg/dl)	79.4±13.4	73.0±9.93	
Apo AI (mg/dl)	95.6±25.3	168±11.3	a

All values are mean ± SEM.
a: $p<0.05$

Table 5.26. Concentrations of lipid and protein components of VLDL and LDL subfractions in 7 male PA patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr
CH	2.72± 0.92	2.02± 0.54	5.15± 1.71	3.44± 0.79	14.1± 4.32	9.51± 1.94	15.3± 2.87	15.7± 2.46	50.3± 5.36	64.7± 9.10	31.1± 6.27	29.0± 7.86
TG	19.9± 6.96	13.8± 3.32	24.3± 7.05	14.7± 5.07	30.1± 6.61	18.4± 4.82	9.63± 1.38	7.20± 1.07	9.46± 1.08	7.75± 1.08	4.7± 1.16	3.40± 0.95
PL	3.79± 1.46	3.18± 0.74	6.37± 2.11	5.55± 1.26	13.8± 3.52	10.5± 1.95	10.6± 1.68	11.2± 1.59	33.8± 5.12	40.4± 5.32	18.5± 3.45	18.7± 5.35
EC	1.93± 0.49	1.47± 0.34	3.74± 1.08	2.58± 0.60	12.3± 3.64	8.80± 2.00	17.5± 3.21	17.4± 2.89	56.1± 5.76	76.4± 11.2	37.5± 7.27	34.9± 9.35
FC	1.57± 0.63	1.15± 0.34	2.92± 1.08	1.91± 0.44	6.70± 2.24	4.27± 0.76	4.93± 1.06	5.32± 0.76	16.8± 2.15	19.3± 2.57	8.76± 2.04	8.19± 2.33
Prot	2.75± 0.97	2.31± 0.68	1.90± 1.47	4.78± 1.03	10.9± 2.93	8.49± 1.57	9.86± 1.28	11.2± 1.64	35.7± 2.27	45.7± 7.42	27.4± 5.36	26.1± 8.15
Apo B	1.62± 0.57	1.07± 0.29	2.74± 0.87	2.74± 0.69	7.63± 2.37	6.08± 1.33	8.78± 1.24	9.65± 1.45				
Mass	30.0± 10.5	21.9± 5.07	42.3± 12.7	29.5± 7.66	73.8± 18.7	50.5± 10.7	52.5± 7.82	52.3± 7.79	152± 15.7	189± 26.4	96.8± 19.1	91.3± 25.8

All concentrations are mean ± SEM (mg/dl).
Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol;
FC: free cholesterol; Prot: protein.

Table 5.27. Composition of VLDL and LDL subfractions in 7 male PA patients and their controls.

	VLDL-1		VLDL-2		VLDL-3		LDL-1		LDL-2		LDL-3	
	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr	PA	Contr
CH	9.49± 0.92	10.4± 1.40	11.7± 0.60	13.7± 2.17	18.1± 0.87	19.7± 1.42	28.4± 1.24	29.7± 0.81	33.0± 0.48	34.2± 0.70	31.9± 0.66	31.2± 1.46
TG	66.3± 1.10	59.7± 4.08	58.1± 0.98	42.9± 6.54	42.0± 1.46	33.6± 3.44	19.3± 2.09	13.9± 0.92	6.30± 0.45	4.17± 0.45 b	4.62± 0.28	5.63± 2.48
PL	11.6± 0.78	16.0± 1.64 a	14.9± 0.68	21.4± 2.94	18.7± 0.25	21.8± 1.19 a	20.1± 0.62	21.8± 0.51	21.8± 0.99	21.5± 0.66	19.4± 0.74	20.1± 0.56
EC	8.05± 0.80	7.77± 1.16	9.40± 0.64	10.2± 1.63	16.5± 1.44	17.8± 1.58	32.6± 1.97	32.6± 1.27	36.9± 0.71	40.1± 1.4	38.8± 1.34	38.0± 1.77
FC	4.70± 0.93	5.76± 0.84	6.07± 0.86	7.65± 1.28	8.28± 0.89	9.14± 0.70	8.98± 0.67	10.3± 0.25	11.0± 0.67	10.3± 0.39	8.83± 0.59	8.56± 0.56
Prot	9.33± 0.35	10.8± 1.04	11.6± 0.52	17.8± 2.44 a	14.5± 0.31	17.7± 0.95 b	19.0± 0.42	21.4± 0.44 b	24.0± 0.89	23.9± 1.09	28.3± 0.57	27.6± 1.03

All values are mean % of total mass ± SEM.

Contr: Controls; CH: cholesterol; TG:triglyceride; PL: phospholipid; EC: esterified cholesterol; FC: free cholesterol; Prot: protein.

a: $p<0.05$; b: $p<0.01$

Table 5.28. Qualitative parameters in 7 male PA patients and their controls

	PA patients	Controls	
%VLDL-1/ ΣVLDL	18.2±3.83	21.0±2.51	
%VLDL-2/ ΣVLDL	27.3±1.65	27.3±1.80	
%VLDL-3/ ΣVLDL	54.5±5.10	51.7±3.20	
%LDL-3/ΣLDL	31.3±3.95	24.8±4.38	
LDL chol/ HDL chol	2.89±0.30	2.17±0.50	
HDL_2/ HDL_3 chol	0.77±0.13	0.73±0.10	
HDL trig/chol	0.27±0.03	0.13±0.03	b
VLDL-1 trig/apoB	14.8±2.66	12.8±2.07	
VLDL-2 trig/apoB	9.61±1.09	5.25±1.25	a
VLDL-3 trig/apoB	4.32±0.29	2.83±0.39	a

All values are mean ± SEM.
chol: cholesterol; trig: triglyceride.
a: $p<0.05$

5.4. DISCUSSION

The number of patients, especially gender specific patients, was relatively small in this study. It was therefore necessary to select closely age-matched controls. Additionally, careful statistical interpretation is required for individual differences, since isolated differences, which are not in agreement with other differences nor consistent in both genders, may be present by chance.

Results presented in this study showed that SLE patients had significantly elevated % LDL-3/ΣLDL mass, which is potentially atherogenic since small dense LDL is associated with atherosclerosis and CHD risk. Other trends contributing to atherogenic potential in SLE patients included raised serum and VLDL triglyceride, LDL/HDL cholesterol and HDL triglyceride/cholesterol ratios, together with depressed HDL cholesterol, HDL_2/HDL_3 cholesterol ratio and serum apo AI.

These differences were particularly apparent in female SLE patients, who had highly significantly elevated %LDL-3/ΣLDL mass, not apparent from serum and LDL cholesterol measurements, and other trends included raised serum and VLDL triglyceride, LDL/HDL cholesterol and HDL triglyceride/cholesterol ratios, and reduced HDL cholesterol, HDL_2/HDL_3 cholesterol ratio and apo AI. Differences between male patients and controls were not dissimilar to the female group; serum and VLDL triglyceride and %LDL-3/ΣLDL mass ratio were elevated and HDL cholesterol and apo AI were reduced.

Previous studies have demonstrated some of this dyslipidaemia in SLE. Elevated VLDL cholesterol and triglyceride and depressed HDL cholesterol and apo AI due to disease activity, and raised serum and VLDL cholesterol and triglyceride due to corticosteroid therapy have been reported in SLE patients (Ilowite et al., 1988). In another study, (Leong et al., 1994), SLE patients on corticosteroid therapy had raised serum cholesterol and triglyceride and LDL, and low HDL, but there was no dyslipidaemia attributed to the active disease. SLE patients on hydroxychloroquine, an antiinflammatory agent used to treat SLE, have been reported to have reduced serum, VLDL LDL and HDL triglyceride and VLDL cholesterol (Hodis et al., 1992). It is apparent therefore that dyslipidaemia in SLE may be dependent on the type of therapy as well as disease activity. In the present study, there were non-significant trends towards raised serum and VLDL triglyceride and reduced HDL cholesterol in SLE patients. Although these trends are similar to those found by Ilowite and Leong in SLE patients, it was not possible to attribute these differences to disease activity or steroid therapy because of the relatively small patient numbers. Patients in this study were stable with little or no disease activity, but some patients were being treated with corticosteroids, hydroxychloroquine or both.

Previous studies have only compared basic lipid and lipoprotein parameters, whereas this study has also demonstrated some important qualitative differences in VLDL and LDL subfractions between SLE patients and controls. In SLE patients, and in females especially, %LDL-3/ΣLDL mass was elevated. Prevalence of small dense LDL is often reported to be associated with elevated triglyceride (Austin and Krauss, 1986; Austin et al., 1988) levels and is believed to be produced by cholesterol ester transfer from LDL and HDL to triglyceride-rich lipoproteins in exchange for triglyceride resulting in cholesterol ester-depleted, triglyceride-rich LDL (Tall, 1986). Triglyceride is hydrolysed from this LDL producing small, dense LDL (Auwerx et al., 1988; Levy et al., 1990; Zambon et al., 1993). Elevated serum and VLDL triglyceride in SLE patients, may contribute to the prevalence of small dense LDL. In females, non-significant increases in triglyceride-enrichment of HDL and LDL-2 are indicative of increased CETP activity. Contradicting this, however, % cholesterol ester in VLDL subfractions was reduced in these patients, a result which appears to be genuine rather than a reflection of triglyceride enrichment, as also cholesterol ester/apo B ratios tended to be lower (data not shown). This finding is difficult to explain, but, as this was not present in PA patients, factors specific to SLE and therapeutic drugs may play a role. However, the elevated %LDL-3/ΣLDL mass persists in SLE patients, significantly so in female SLE patients, when they are matched with controls for serum triglyceride, suggesting that other factors specific to the disease may be responsible.

Explanations for dyslipidaemia in SLE in previous studies have included the role of mediators of inflammation and corticosteroids on lipoprotein metabolism. Mediators of inflammation such as interleukin-1 and tumour necrosis factor have been reported to inhibit LPL activity (Beutler et al., 1985; Fried and Zechner, 1989; Ogawa et al., 1989). These cytokines, elevated in SLE could inhibit LPL activity leading to raised VLDL and reduced HDL. In addition, VLDL production is stimulated by steroids. The explanation for an elevated proportion of LDL-3/ΣLDL in SLE patients is far less clear, although elevated triglyceride, mediators of inflammation and steroids could all play a role.

In conclusion, SLE patients had an atherogenic lipoprotein profile including raised %LDL-3/ΣLDL mass, a parameter which was not apparent from simple lipoprotein measurements. This is clearly a preliminary study which warrants further investigation into the effects of disease activity and antiinflammatory drug therapy on the LDL profile in SLE patients.

Results comparing all PA patients and controls, showed that patients had significantly reduced HDL, HDL_2 and HDL_3 cholesterol and apo AI, significantly raised LDL/HDL cholesterol and HDL triglyceride/cholesterol ratios and a non-significant increase in %LDL-3/ΣLDL mass ratio compared to controls ($p=0.06$), contributing to an atherogenic lipoprotein profile. Similarly, female PA patients had significantly reduced HDL cholesterol and elevated LDL-3 mass and cholesterol and %LDL-3/ΣLDL despite having slightly lower serum and VLDL triglyceride. Male patients also had significantly lower HDL cholesterol, and in addition, they had significantly lower HDL_3 cholesterol, apo AI and raised HDL triglyceride/cholesterol ratio compared to controls. Furthermore, both male and female patients tended to have lower levels of serum and LDL cholesterol. Although male patients had substantially, but non-significantly, higher %LDL-3/ΣLDL mass, unlike with the female group, this may be due to elevated serum and VLDL triglyceride and significantly raised triglyceride/apoB ratio in VLDL-2 and -3, contributing to the formation of small dense LDL.

Lazarevic and co-workers (1992) reported that patients with severe PA had reduced serum, LDL and HDL cholesterol and serum triglyceride compared to patients with mild activity, suggesting that disease activity was responsible for hypocholesterolaemia in PA patients. Similarly, in the present study, PA patients, most of whom had active disease, showed signs of hypocholesterolaemia with significantly lower HDL cholesterol and non-significant reductions in serum and LDL cholesterol compared to controls.

Depressed levels of HDL, as described for SLE patients, may be due to inhibition of

LPL activity by cytokines. It is interesting to note that while triglycerides were lower in female PA patients, there were non-significant increases in triglyceride-enrichment of HDL and LDL-2, indicative of enhanced CETP activity. None of the patients were on steroid therapy or any therapy that may affect lipid metabolism, suggesting that the prevalence of small dense LDL in female patients may be due to mediators of inflammation specific to the disease, although the mechanism is unclear.

For both SLE and PA patients percentage LDL-3/ΣLDL mass ratio was significantly raised, particularly in the female groups, and this difference was not associated with elevated triglyceride levels. However, in both the male groups this difference did not achieve significance, which may well be due to small patient numbers. Reduced HDL cholesterol levels compared to controls were apparent in both patient groups. Whereas this was a trend in SLE patients, the difference was significant for PA patients. SLE patients tended to have elevated serum and VLDL triglyceride levels in both males and females, whereas, in PA patients this trend was only observed in the male group. Furthermore, reduced serum and LDL cholesterol was apparent in male and female PA patients, a trend not seen in SLE patients. Perhaps similarities in dyslipidaemia in SLE and PA patients are caused by mediators of inflammation, whereas differences are caused by the different activities of the disease in the patient groups, as well as the influence of therapy in the SLE group.

The prevalence of reduced HDL cholesterol and elevated proportion of small dense LDL in PA and SLE patients have atherogenic implications which may contribute to CHD risk.

CHAPTER 6

ANTI-XANTHINE OXIDASE ANTIBODIES IN HEALTHY SUBJECTS

6.1. INTRODUCTION

In a previous study (Harrison et al.,1990), IgM anti-BXO antibodies were found to be present in the serum of all subjects examined and levels were raised in patients who had suffered an MI, compared to controls. There were no such differences in IgG and IgA anti-BXO antibodies. Such antibodies could originate in response to ingested bovine milk (Oster et al 1971; Rzucildo et al.,1979; Deeth 1983), or they could be autoantibodies in response to endogenous XO, known to be present in the capillary endothelium of a variety of tissues (Jarasch et al., 1986).

In order to learn more about the distribution and origin of anti-XO antibodies in normal subjects, IgG and IgM antibodies to BXO and HXO were compared in serum samples obtained from carefully matched groups of human donors.

6.2. METHOD

6.2.1. Study population

Serum samples were collected from the Bath Clinic. Subjects were normal individuals, attending for routine health screening, with neither present nor past history of CHD (Table 6.1).

	MALES	FEMALES
NUMBER	129	129
AGE ± SEM (yrs)	46.7 ± 0.93 (20-72)	46.8 ± 0.92 (19-72)

Table 6.1: demographic data for males and females.

6.2.2. Measurements

The anti-BXO IgM and IgG antibodies were measured by ELISA using freshly prepared bovine XO prepared by the method of Nakamura and Yamazaki (1982) (supplied by J.Khan, Bath University). The anti-HXO IgM and anti-HXO IgG results were obtained from A.Gleeson (Bath University). Antibody levels, measured as a percentage of a high titre standard serum (A1), were converted to actual concentration of antibody by using values of total antibody concentration and XO-specific antibody concentrations in the standard serum (supplied by M.Benboubetra, Bath University). These values are as follows: 3.0 mg/ml total IgM, 15.04 mg/ml total IgG, 53.0 μg/ml BXO-specific IgM, 64.0 μg/ml HXO-specific IgM, 8.0 μg/ml BXO-specific IgG, and 4.2 μg/ml HXO-specific IgG. Comparisons of anti-BXO and anti-HXO IgM and IgG antibodies were made using all the serum samples. In addition comparisons were made between males and females.

6.2.3. Statistics

Comparisons were made using the Kolmogorov-Smirnov test.

6.3. RESULTS

Table 6.2 shows the mean concentrations of IgM and IgG class anti-BXO and anti-HXO antibodies in the serum samples from 258 human subjects, as well as their concentration expressed as a percentage of total IgM and IgG antibody level. Distributions profiles of the concentrations of IgM anti-BXO and anti-HXO antibodies and the concentrations of IgG anti-BXO and anti-HXO antibodies are shown in Fig. 6.1 and Fig. 6.2 respectively. IgM anti-HXO antibody levels were significantly higher than IgM anti-BXO antibody levels and IgG anti-BXO antibody levels were significantly higher than IgG anti-HXO antibody levels. However, as shown in Table 6.2, IgM anti-BXO and anti-HXO antibodies were present at higher levels than the corresponding IgG levels, and were also higher in terms of percentage of total antibody of its own class.

Table 6.2. Levels of anti-XO antibodies in 258 human serum samples

	IgM		IgG	
Specific antibody	anti-BXO	anti-HXO	anti-BXO	anti-HXO
Number of subjects	258	258	258	258
Age in years (mean ± SEM)	46.8±0.64	46.8±0.64	46.8±0.64	46.8±0.64
Mean titre (mean ± SEM)	24.1±1.23 (μg/ml)	42.6±2.01 (μg/ml)	7.27±0.57 (μg/ml)	2.68±0.14 (μg/ml)
Mean % of class total	0.80	1.42	0.05	0.02
Dmax	0.353 (35.3%)		0.376 (37.6%)	
p value	<0.001		<0.001	

Dmax is the maximum cumulative difference for this Table and subsequent Tables.

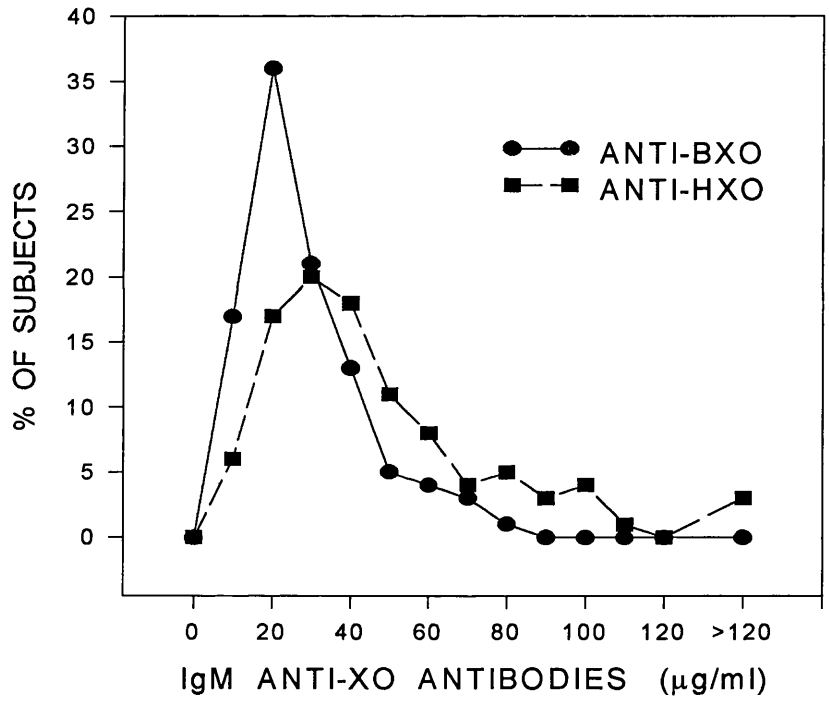


Fig. 6.1. Comparison of IgM anti-BXO and anti-HXO antibody levels in 258 males and females. p<0.001.

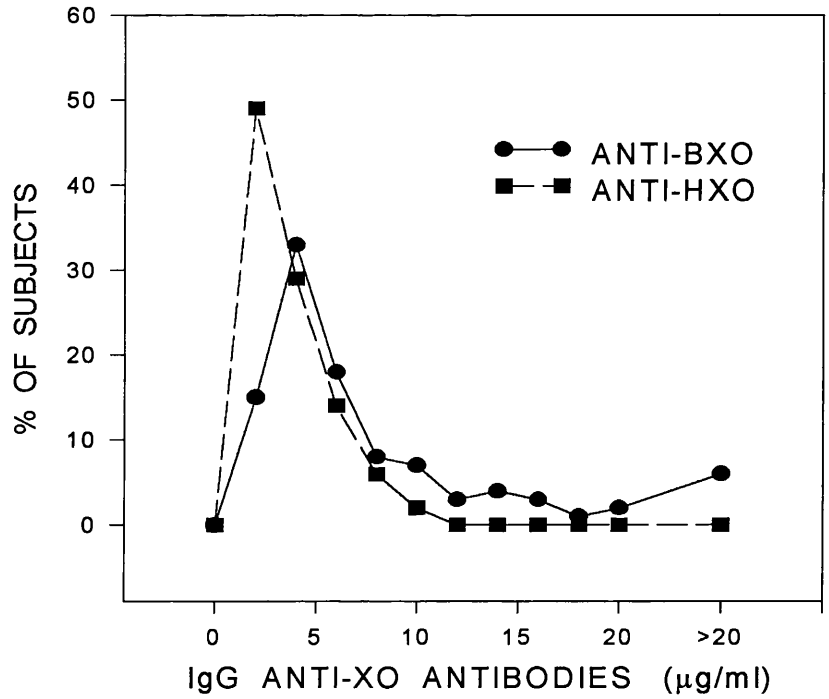


Fig. 6.2. Comparison of IgG anti-BXO and anti-HXO antibody levels in 258 males and females. $p<0.001$.

Comparisons of IgM and IgG anti-BXO antibodies for males and females, above and below 50 years, are shown in Tables 6.3 and 6.4 respectively. The corresponding comparisons for anti-HXO antibodies are shown in Tables 6.5 and 6.6. The distribution profiles show that IgM anti-BXO antibodies were significantly higher in females compared to males (Fig. 6.3), particularly in subjects below 50 years (Fig. 6.4), and higher in females below 50 years compared to females above 50 years (Fig. 6.5). There were no differences in these antibodies between males and females above 50 years, or between males above and below 50 years. There were also no differences in IgG anti-BXO antibodies between the aforementioned subject groups. An identical pattern of results was observed for IgM and IgG anti-HXO antibodies (Tables 6.5 and 6.6).

Table 6.3. Levels of IgM anti-BXO antibodies in male and female subjects.

	IgM anti-BXO antibodies									
	Male total	Fem total	Male <50 years	Fem <50 years	Male ≥50 years	Fem ≥50 years	Male <50 years	Male ≥50 years	Fem <50 years	Fem ≥50 years
N	129	129	79	79	50	50	79	50	79	50
Age (mean± SEM) yrs	46.8 ± 0.91	46.8 ± O.91	40.0 ± 0.68	40.0 ± 0.68	57.8 ± 0.75	57.8 ± 0.75	40.0 ± 0.68	57.8 ± 0.75	40.0 ± 0.68	57.8 ± 0.75
Mean Titre (mean± SEM) µg/ml	21.0 ± 1.40	27.2 ± 2.07	22.8 ± 2.05	32.5 ± 3.03	18.0 ± 1.53	18.9 ± 1.8	22.8 ± 2.05	18.0 ± 1.53	32.5 ± 3.03	18.9 ± 1.82
Dmax	0.21		0.29		0.10		0.14		0.44	
p value	<0.01		<0.005		>0.1		>0.1		<0.001	

Fem: Female, for this Table and subsequent Tables.

Table 6.4. Levels of IgG anti-BXO antibodies in male and female subjects.

	IgG anti-BXO antibodies									
	Male total	Fem total	Male <50 years	Fem <50 years	Male ≥50 years	Fem ≥50 years	Male <50 years	Male ≥50 years	Fem <50 years	Fem ≥50 years
N	129	129	79	79	50	50	79	50	79	50
Age (mean± SEM) yrs	46.8 ± 0.91	46.8 ± O.91	40.0 ± 0.68	40.0 ± 0.68	57.8 ± 0.75	57.8 ± 0.75	40.0 ± 0.68	57.8 ± 0.75	40.0 ± 0.68	57.8 ± 0.75
Mean Titre (mean± SEM) µg/ml	7.18 ± 0.93	7.35 ± 0.67	6.94 ± 1.19	8.00 ± 1.98	7.51 ± 1.51	6.37 ± 0.92	6.94 ± 1.19	7.51 ± 1.51	8.00 ± 1.98	6.37 ± 0.92
Dmax	0.10		0.15		0.10		0.05		0.17	
p value	>0.1		>0.1		>0.1		>0.1		>0.1	

Table 6.5. Levels of IgM anti-HXO antibodies in male and female subjects.

	IgM anti-HXO antibodies									
	Male total	Fem total	Male <50 years	Fem <50 years	Male ≥50 years	Fem ≥50 years	Male <50 years	Male ≥50 years	Fem <50 years	Fem ≥50 years
N	129	129	79	79	50	50	79	50	79	50
Age (mean± SEM) yrs	46.8 ± 0.91	46.8 ± 0.91	40.0 ± 0.68	40.0 ± 0.68	57.8 ± 0.75	57.8 ± 0.75	40.0 ± 0.68	57.8 ± 0.75	40.0 ± 0.68	57.8 ± 0.75
Mean Titre (mean± SEM) µg/ml	38.6 ± 2.61	46.7 ± 3.07	39.0 ± 3.42	51.8 ± 4.41	37.8 ± 4.04	38.9 ± 3.54	39.0 ± 3.42	37.8 ± 4.04	51.8 ± 4.41	38.9 ± 3.54
Dmax	0.16		0.24		0.10		0.09		0.24	
p value	<0.05		<0.025		>0.1		>0.1		<0.05	

Table 6.6. Levels of IgG anti-HXO antibodies in male and female subjects.

	IgG anti-HXO antibodies									
	Male total	Fem total	Male <50 years	Fem <50 years	Male ≥50 years	Fem ≥50 years	Male <50 years	Male ≥50 years	Fem <50 years	Fem ≥50 years
N	129	129	79	79	50	50	79	50	79	50
Age (mean± SEM) yrs	46.8 ± 0.91	46.8 ± O.91	40.0 ± 0.68	40.0 ± 0.68	57.8 ± 0.75	57.8 ± 0.75	40.0 ± 0.68	57.8 ± 0.75	40.0 ± 0.68	57.8 ± 0.75
Mean Titre (mean± SEM) µg/ml	2.64 ± 0.21	2.72 ± 0.17	2.02 ± 0.18	2.64 ± 0.22	3.58 ± 0.45	2.72 ± 0.26	2.02 ± 0.18	3.58 ± 0.45	2.64 ± 0.22	2.72 ± 0.26
Dmax	0.13		0.17		0.15		0.21		0.10	
p value	>0.1		>0.1		>0.1		>0.1		>0.1	

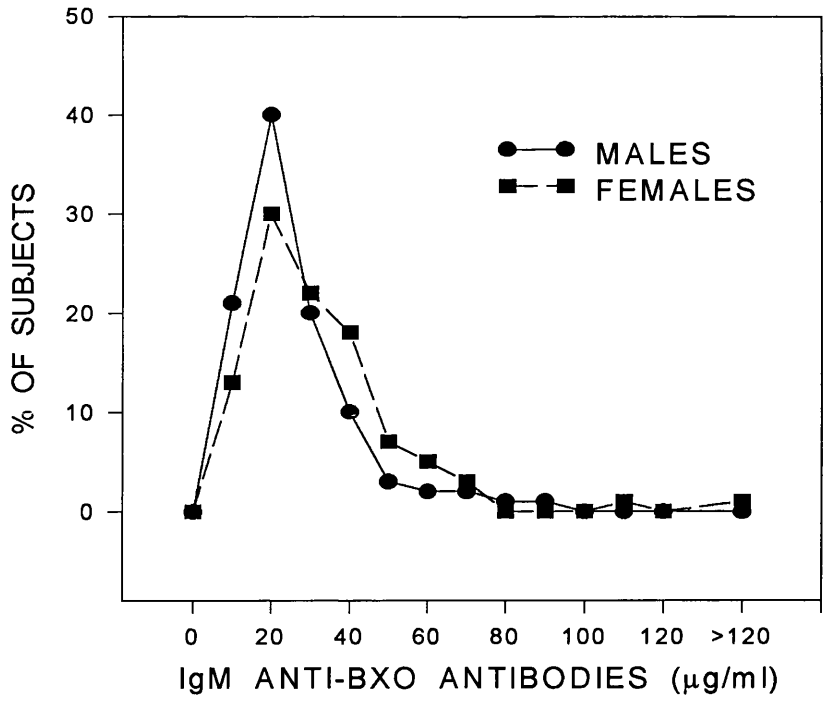


Fig. 6.3. Comparisons of IgM anti-BXO antibody levels in 129 males and age-matched females. $p<0.01$.

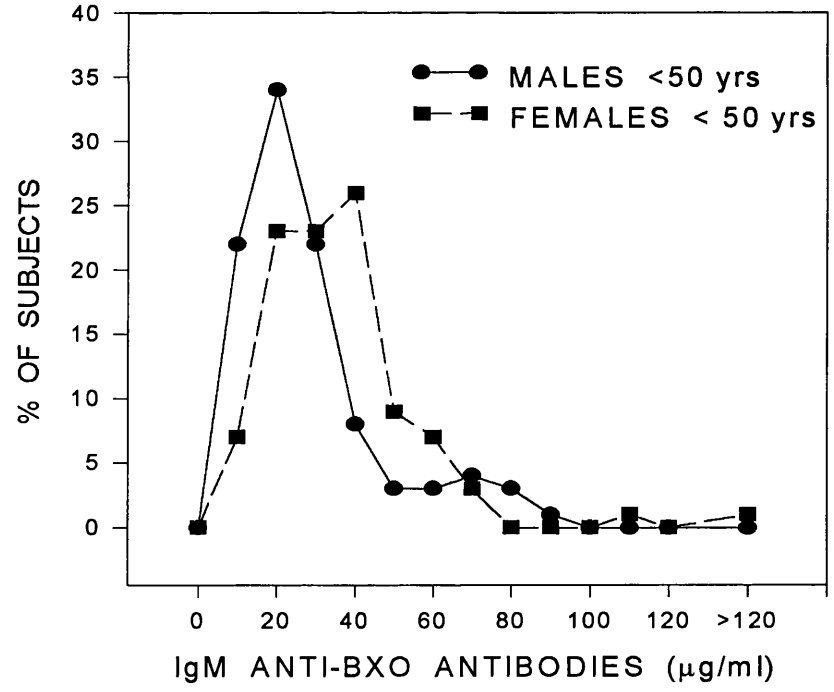


Fig. 6.4. Comparison of IgM anti BXO antibodies in 79 males and females under 50 years of age. $p<0.005$.

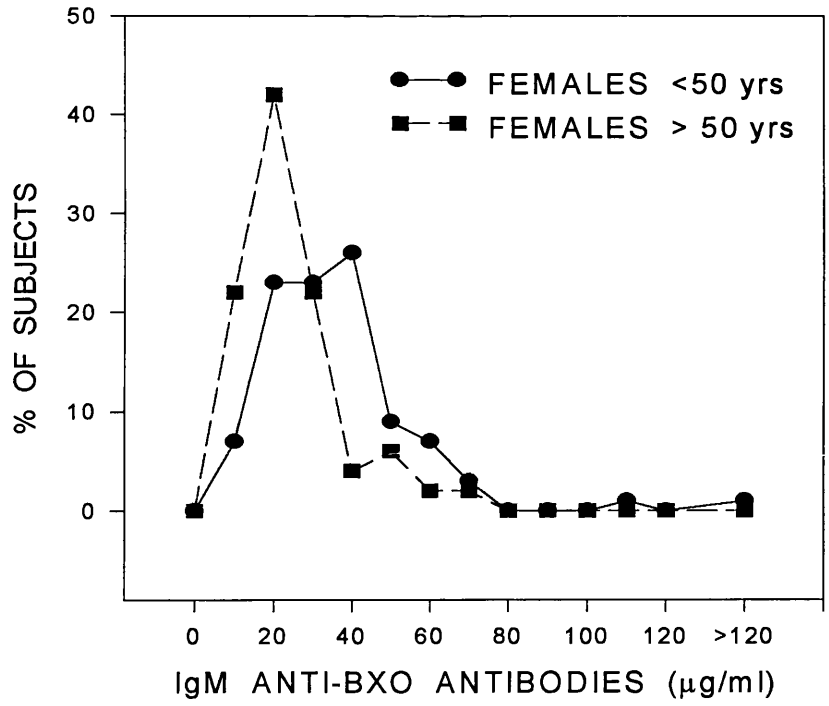


Fig. 6.5. Comparisons of IgM anti-BXO antibody levels in females above and below 50 years of age. $p<0.001$.

6.4. DISCUSSION

The levels of IgM anti-XO antibodies found in normal human serum was surprisingly high, with IgM class anti-HXO antibodies (mean titre 42.6 µg/ml) making up 1.4 % of total IgM. In view of the proposed involvement of XO in the generation of reactive oxygen species during ischaemia-reperfusion (Sussman and Buckley, 1990), the origin and role of these antibodies is clearly of interest.

Concerning their origin, levels of IgM anti-HXO antibodies, were significantly ($p<0.001$) higher than those of IgM anti-BXO antibodies suggesting that endogenous HXO, rather than ingested BXO is the immunogen.

In the course of initial studies, it quickly became apparent that female subjects in general, show higher titres than do males, and the subject population was accordingly chosen to allow precise gender and age-matching. Comparison of IgM anti-BXO antibody titres in 129 male and 129 female subjects showed those of the latter to be significantly ($p<0.01$) higher; a difference that can clearly be attributed to the higher titres in women under 50 years, which are not only higher than those of age-matched men ($p<0.005$) but also than those of older women ($p<0.001$). Men, on the other hand, showed no significant age-dependent differences. Similar results were obtained for anti-HXO antibodies.

Similar findings have been reported by Lewis and Ng (1991), who compared serum IgM anti-BXO antibody levels in 105 males and 106 females, all under 50 years, and found titres in the females to be significantly higher ($p<0.01$). Antibody levels in 54 females under 50 years were significantly ($p<0.005$) higher than in 47 older females. Lewis and Ng (1991) suggested that higher titres in younger women may occur because pre-menopausal females might be cyclically re-immunised by XO released during ischaemic events preceding menstruation. This, of course, assumes that the immunogen is HXO rather than BXO, an assumption that was reinforced by the fact (Lewis and Ng, 1991) that no differences in titres were observed between European and Chinese subjects, despite marked differences in bovine milk product consumption between the two population groups.

In contrast to IgM anti-XO class antibodies, those of IgG class showed no variations with gender with gender and age and represented a much lower proportion of their total class. Levels of IgG anti-HXO antibodies (mean titre 2.68 μg/ml) represent only 0.02% total IgG while anti-BXO antibodies (mean titre 7.27 μg/ml) make up 0.05% of total IgG. Anti-BXO antibody levels determined in the present study are of the same order as those represented by Ng et al. (1990) who found average values for IgM anti-BXO antibodies of 11.9 μg/ml (cf. 24.1μg/ml, Table 6.2) and for IgG anti-BXO antibodies of 14.7 μg/ml (cf. 7.27 μg/ml, Table 6.2). Lewis and Ng (1991), like myself, found no sex or age dependency of IgG anti-BXO levels.

The work of Bruder et al. (1984) should also be considered. In striking contrast to both the present data and those of Ng et al. (1990), they found only IgG class antibodies in human serum. This conclusion was based on affinity purification of anti-XO antibodies from serum, followed by analyses in SDS-PAGE, when γ and but not μ antibody chains were detected. Using an ELISA, they compared anti-BXO and anti-HXO antibody levels in the sera of 74 human subjects, based on detection of antibodies by protein A, which they took to be specific for IgG. Their findings, based on apparent IgG, show many similarities to my conclusions with IgM anti-XO antibodies. Bruder et al. (1984) also found anti-HXO class antibodies to be higher than those of anti-BXO, although their absolute levels were higher than mine. Their values of 0.2-1.5 % and 0.5-3.0 % total IgG for anti-BXO and anti-HXO antibodies respectively represent 30-226 μg/ml and 75-451 μg/ml (cf. Table 6.2). The two studies could be partially reconciled if the protein A-based ELISA of Bruder and co-workers had, in fact, been detecting IgM, possibly in addition to IgG. Binding of protein A to human IgM can indeed occur and the above explanation may be valid.

My findings of high levels of IgM anti-XO antibodies in human serum clearly raise many questions. The suggestion, from both the present study and that of Lewis and Ng

(1991), is that the antibodies arise in response to endogenous HXO, rather than to ingested BXO. In this case, the autoantibodies could be seen as protective, acting to neutralise and clear pathologically-derived XO from the blood and tissues. The presence of elevated IgM anti-XO antibodies in MI patients (Harrison et al.,1990) can be seen as consistent with this idea. Harrison et al. (1990) presented evidence that elevated anti-XO antibody levels are not merely a consequence of the MI itself. They can be seen as a result of chronic lesions of capillary endothelium exposing XO to the immune system, possibly over a period of many months prior to clinical manifestations (Harrison et al.,1990). In this context, raised levels of IgM anti-XO antibodies in human serum would be seen as a marker of CHD, rather than as a positive risk factor. A protective role for IgM anti-XO antibodies is consistent with the sex- and age- linked differences reported in the present Chapter. Younger women have a lower incidence of myocardial infarction than men of similar age (Heller, 1978). This advantage is largely lost after the age of the onset of the menopause, when, according to my present findings, levels of IgM anti-XO antibodies fell significantly. It is relevant, at this stage to refer ahead to Chapter 7, in which it is shown that patients dying within 6 months after an MI have significantly higher IgM anti-XO levels than 'survivors'. In the absence of any positive correlation between antibody titre and extent of MI (as judged by CPK levels; Chapter 7, Table 7.14), it can be agreed that those MI patients most at risk of death are those with most extensive capillary endothelial damage as evidenced by higher IgM anti-XO antibody levels.

The situation regarding IgG class anti-XO antibodies in human serum is quite different from that for those of IgM class. Levels of the former were found to be lower (Table 6.2), although Ng et al. (1990) reported similar average titres for anti-BXO antibodies of IgG (14.7 μg/ml) and IgM (11.9μg/ml) class. Neither the present study or that of Lewis and Ng (1991) found any association of IgG anti-XO levels with age or gender, nor did Harrison et al. (1990) find any correlation of such levels with MI. Moreover, levels of IgG anti-BXO antibodies are significantly ($p<0.001$) higher than IgG anti-HXO antibodies (Table 6.2); the reverse of that found in the case of IgM antibodies. There are no ready explanations for the differences between IgG and IgM class antibodies. If it is assumed that the basic situation involves IgM anti-HXO antibodies with a protective function, then possibly one could imagine that B-lymphocyte clones providing strongly cross-reactive antibodies could be induced to class switch from IgM and IgG by exposure to ingested BXO fragments. Clearly such an explanation is highly speculative but is offered in the absence of a better.

CHAPTER 7

ANTI-XANTHINE OXIDASE ANTIBODIES AND MYOCARDIAL INFARCTION

7.1. INTRODUCTION

Harrison et al. (1990) found raised levels of IgM anti-BXO levels in patients with MI compared to controls. They also noted indications that levels were higher in patients who had died within 6 months of the MI compared to survivors.

The aim of this study was to investigate this latter suggestion and further to assess the extent to which antibody levels simply reflect the severity of the MI, as judged by serum creatinine phosphokinase (CPK) levels. Indirect evidence suggests that the endothelium of hypercholesterolaemic vessels is a source of ROS, probably via XO activation (Ohara et al., 1993). In view of the associations between atherosclerotic lesions and elevated lipid levels, antibody levels were correlated with serum and lipoprotein cholesterol and triglyceride.

7.2. METHODS

7.2.1. Study population

Serum samples were collected from subjects (MI patients and controls), all of whom had been admitted to the Coronary Care Unit (CCU), Bath Royal United Hospital. Controls had no signs of heart disease or family history of heart disease. 'Deaths' refer to patients who died within 6 months after an MI and 'survivors' refer to patients who survived at least 6 months after an MI. Some controls, used in an early study were from a study clinic in the community in Trowbridge (supplied by Dr. J.Lloyd, Royal United Hospital, Bath); they were healthy volunteers who had not been admitted to the CCU. All subjects were male.

7.2.2. Measurements

All blood samples were taken from subjects within 24 hours of admission to the CCU, and serum was stored at -70°C prior to assay. Serum samples from the Trowbridge

study were also stored at −70°C prior to assay. Anti-BXO IgM antibodies were measured in each sample and expressed as a percentage of a high titre standard serum (A1). The enzyme used in the ELISA was supplied by Biozyme Ltd. CPK, cholesterol and triglyceride levels were obtained from the CCU admission file where possible. For a randomly selected group of 60 MI patients, cholesterol and triglyceride were also measured in plasma and serum lipoproteins (VLDL, LDL, HDL, HDL_2, and HDL_3) for correlation analysis.

7.2.3. Statistics

Comparisons involving antibody levels and CPK levels were made by using the Kolmogorov-Smirnoff test. Comparisons involving cholesterol and triglyceride (log-transformed) were made using an unpaired Student's t-test. Correlations between antibody levels and CPK, lipid and lipoprotein measurements were made using Spearman's rank correlation test. All values are mean ± SEM.

7.3. RESULTS

7.3.1. Comparison of antibody levels between MI patients and controls (36 Trowbridge controls and 16 CCU controls).

IgM anti-BXO antibody levels were significantly higher in controls compared to MI patients (Table 7.1., Fig. 7.1) At the point of maximum cumulative difference 48% of MI patients had been represented compared to 21% of the controls.

Table 7.1. Comparison of antibody levels between 52 MI patients and 52 age-matched controls (36 Trowbridge controls and 16 CCU controls).

	MI patients	Controls
NUMBER	52	52
AGE (yrs) ± SEM	56.1±1.3	56.1±1.3
Mean titres ± SEM	18.3±1.78	25.0±2.30
Dmax	0.269 (26.9%)	
p value	$p<0.05$	

Titres are mean ± SEM (% of A1 standard).
Dmax is the maximum cumulative difference for this Table and subsequent Tables.

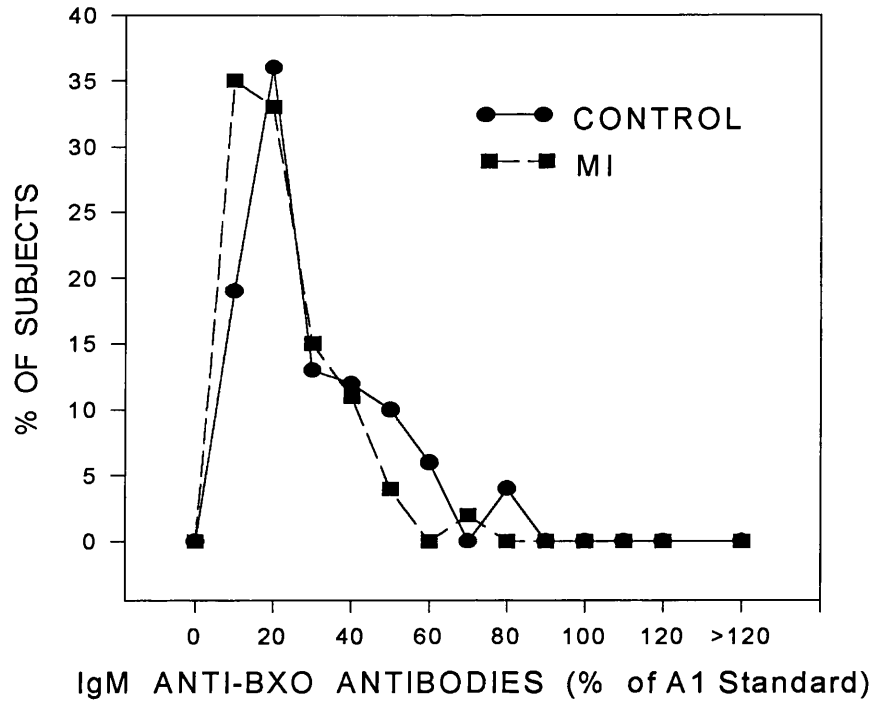


Fig. 7.1. Comparison of IgM anti-BXO antibody levels in 52 MI patients and their age-matched controls. $p<0.05$.

7.3.2. Comparison of antibody levels between Trowbridge controls and age-matched CCU controls

In a previous study (Harrison et al., 1990), where it was reported that MI patients had significantly higher IgM anti-BXO antibody levels than controls, the entire control group was selected from the CCU. In order to investigate whether the two control groups (Trowbridge controls and CCU controls) differed in terms of IgM anti-BXO antibody levels, a comparison was made between 36 Trowbridge controls and 36 age-matched CCU controls. No significant differences were found between IgM anti-BXO antibody levels in Trowbridge controls and CCU controls (Table 7.2, Fig. 7.2). However, the mean antibody titre was higher in the Trowbridge control group and at the point of maximum cumulative difference 58% of CCU controls had been represented compared to 36% of the Trowbridge controls. A Dmax difference of 33% was needed for statistical significance.

Table 7.2. Comparison of antibody levels between Trowbridge controls and age-matched CCU controls.

	Trowbridge controls	CCU controls
NUMBER	36	36
AGE (yrs) ± SEM	59.3±1.2	59.3±1.2
Mean titres ± SEM	45.0±4.00	37.4±3.60
Dmax	0.220 (22%)	
p value	>0.1	

Titres are mean ± SEM (% of A1 standard).

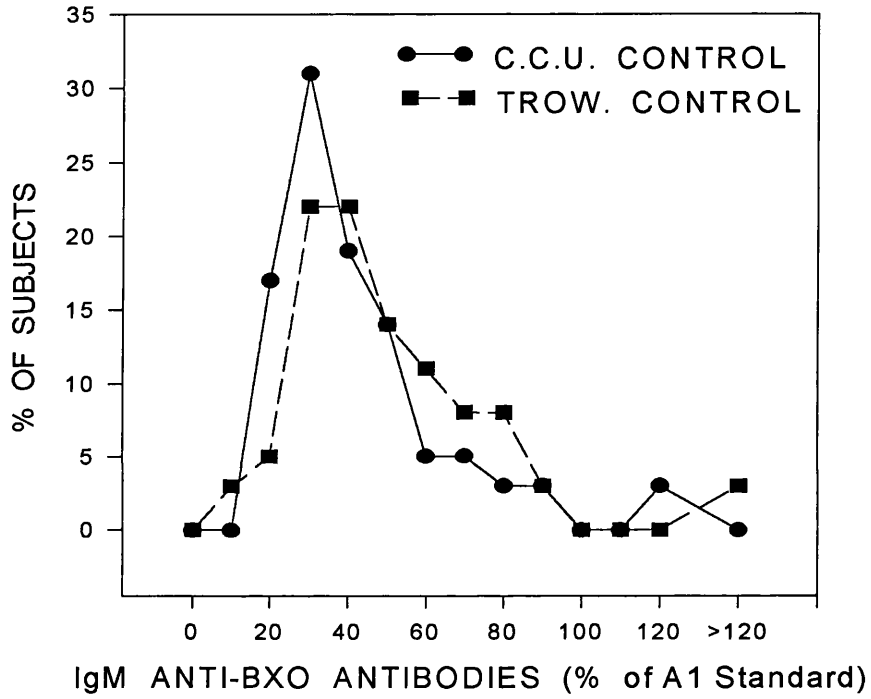


Fig. 7.2. Comparisons of IgM anti-BXO antibody levels in 36 controls from the CCU and 36 age-matched controls. p>0.1.

7.3.3. Comparison of antibody levels between MI patients and controls.

In view of the results of section 7.3.2, above, CCU controls were considered to be more appropriate than Trowbridge controls (see Discussion). Accordingly a comparison of IgM anti-BXO antibody levels was made between MI patients and CCU controls. Again, there was no significant difference between the two subject groups (Table 7.3, Fig. 7.3). However this time, MI patients had a higher mean titre than the control group, and at the

point of maximum cumulative difference, 53% of the control group had been represented compared to 42% of the MI patients. A Dmax value of 13.4% would have been required for the difference to have reached statistical significance.

Table 7.3. Comparison of antibody levels between 311 MI patients and 154 age-matched controls.

	MI patients	Controls
NUMBER	311	154
AGE (yrs) ± SEM	53.8 ± 0.51	51.7 ± 0.89
Mean titres ± SEM	31.1±1.30	26.6±1.29
Dmax	0.105 (10.5%)	
p value	>0.1	

Titres are mean ± SEM (% of A1 standard).

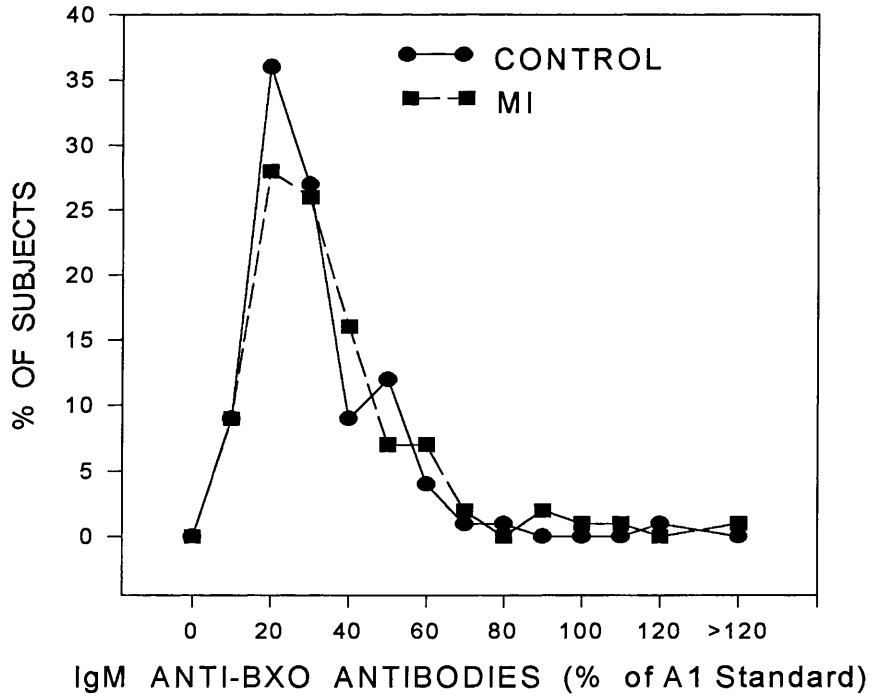


Fig. 7.3. Comparison of IgM anti-BXO antibodies in 311 MI patients and 154 age-matched controls. p>0.1.

7.3.4. Comparison of antibody levels between patients who had died within 6 months from a MI ('deaths') and patients who had survived at least 6 months after a MI ('survivors').

Table 7.4 and Fig. 7.4 show that IgM anti-BXO antibody levels were significantly higher in 'deaths' than in 'survivors'. At the point of maximum cumulative difference 30% of 'deaths' had been represented compared to 47% of 'survivors'.

Table 7.4. Comparison of antibody levels between 94 patients who had died within 6 months from a MI and 234 age-matched patients who had survived at least 6 months after a MI.

	Deaths	Survivors
NUMBER	94	234
Previous MI (% of patients)	4.3	6.0
AGE (yrs) ± SEM	66.3 ±0.95	64.2 ±0.51
Mean titres ± SEM	29.5±2.04	27.4±1.43
Dmax	0.172	
p value	<0.05	

Titres are mean ± SEM (% of A1 standard).

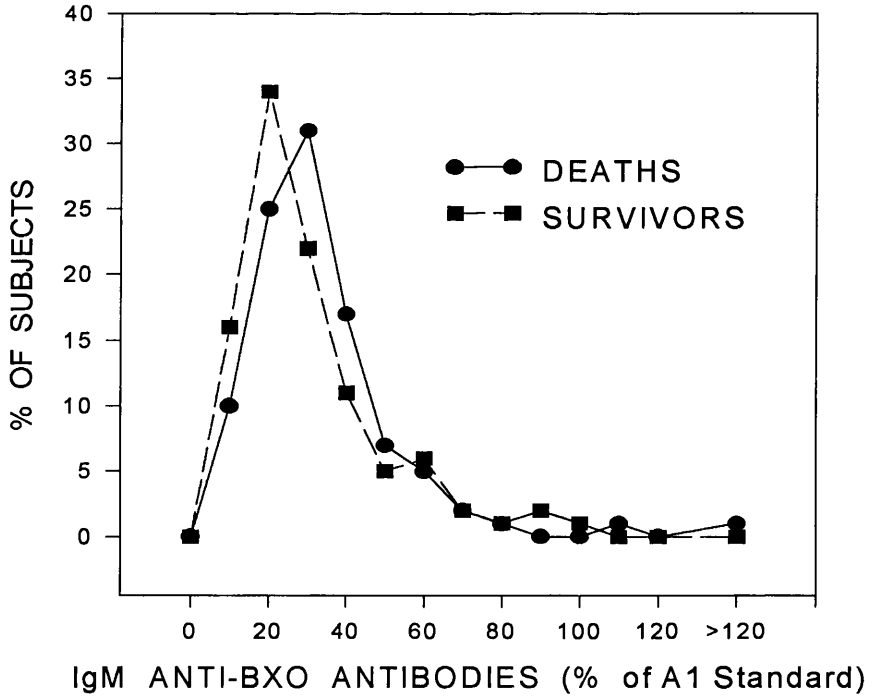


Fig. 7.4. Comparisons of IgM anti-BXO antibody levels in 94 patients who died within 6 months of an MI and in 234 age-matched patients who survived at least 6 months. P<0.05.

7.3.5. Comparison of antibody levels between patients who had died within 6 months from a MI and controls.

In view of 'deaths' having significantly higher antibody titres than 'survivors', and MI patients having higher titres than controls (although differences did not reach significance), it seemed logical that 'deaths' would have higher antibody titres than controls. The latter comparison is shown in Table 7.5.

Table 7.5. A comparison of anti-BXO IgM antibodies between 47 patients who had died from a MI (deaths) and 79 age-matched controls.

	Deaths	Controls
NUMBER	47	79
AGE (yrs) ± SEM	59.3 ±1.16	60.2 ± 0.72
Mean titres ± SEM	31.7±3.55	25.1±1.73
Dmax	0.179 (17.9%)	
p value	>0.1	

Titres are mean ± SEM (% of A1 standard).

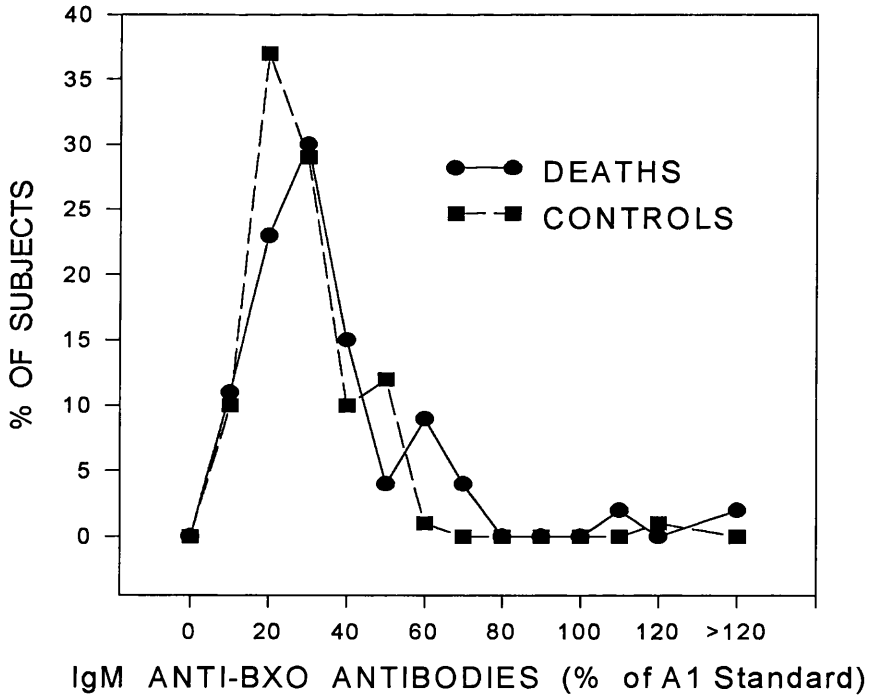


Fig. 7.5. Comparisons of IgM anti-BXO antibody levels in 47 patients who have died within 6 months of a MI and 79 age-matched controls. p>0.1.

Table 7.5 and Fig. 7.5 confirm that 'deaths' had higher antibody titres than controls, although this difference did not reach statistical significance. At the point of maximum cumulative difference, 35% of 'deaths' had been represented compared to 52% of the controls. A Dmax of 25% would have been required for the difference to have reached statistical significance.

7.3.6. Plasma and lipoprotein cholesterol and triglyceride concentrations and their relationship with IgM anti-BXO antibodies.

A comparison of plasma cholesterol and triglyceride concentrations was made between MI patients and controls (Table 7.6), between 'deaths' and 'survivors' (Table 7.7), and between 'deaths' and controls (Table 7.8). MI patients had significantly elevated cholesterol levels compared to control patients. There were no significant differences in cholesterol and triglyceride between the other subject groups.

Table 7.6. Comparisons of cholesterol and triglyceride levels between MI patients and controls.

	MI patients	Controls	p value
Cholesterol (mmol/l) mean ± SEM	6.29±0.10 (n=270)	5.67±0.14 (n=82)	0.002
Triglyceride (mmol/l) mean ± SEM	2.47±1.65 (n=232)	2.17± 1.17 (n=71)	>0.1

Table 7.7. Comparisons of cholesterol and triglyceride levels between 'deaths' and 'survivors'.

	Deaths	Survivors	p value
Cholesterol (mmol/l) mean ± SEM	5.87±0.17 (n=64)	6.23±0.11 (n=179)	0.085
Triglyceride (mmol/l) mean ± SEM	2.01±1.18 (n=50)	2.38±1.55 (n=148)	>0.1

Table 7.8. Comparisons of cholesterol and triglyceride levels between 'deaths' and controls.

	Deaths	Controls	p value
Cholesterol (mmol/l) mean ± SEM	5.91±0.21 (n=34)	5.53±0.19 (n=39)	>0.1
Triglyceride (mmol/l) mean ± SEM	1.90±0.94 (n=28)	2.10±1.29 (n=36)	>0.1

Correlations of lipid levels and antibody levels in 'deaths', 'survivors', and controls, as individual groups and as one collective group, are shown in Table 7.9. In addition, cholesterol and triglyceride levels for VLDL, LDL, HDL, HDL_2 and HDL_3 were correlated with antibody levels in a group of MI patients (Table 7.10). There were no significant correlations between IgM anti-BXO antibody levels and lipid and lipoprotein levels in any of the subject groups.

Table 7.9. Correlations between IgM anti-BXO antibodies and plasma cholesterol and triglyceride levels.

Antibody levels in the subjects below	Cholesterol	Triglyceride
Deaths	Rs=0.002 p >0.1 (n=64)	Rs= –0.26 p=0.067 (n=50)
Survivors	Rs=0.003 p >0.1 (n=179)	Rs=0.059 p >0.1 (n=148)
Controls	Rs=0.119 p >0.1 (n=82)	Rs=0.04 p >0.1 (n=71)
Total (deaths, survivors, controls)	Rs=0.023 p >0.1 (n=325)	Rs=0.001 p >0.1 (n=269)

Rs: Spearman's rank correlation coefficient.

Table 7.10. Correlations between IgM anti-BXO antibody levels and lipids and lipoprotein levels in 60 MI patients.

Lipid/ lipoprotein	(Rs)	p value
Serum cholesterol	0.183	>0.1
Serum triglyceride	–0.093	>0.1
VLDL cholesterol	–0.027	>0.1
VLDL triglyceride	–0.078	>0.1
HDL cholesterol	0.143	>0.1
HDL triglyceride	–0.020	>0.1
HDL_2 cholesterol	0.015	>0.1
HDL_2 triglyceride	0.156	>0.1
HDL_3 cholesterol	0.127	>0.1
HDL_3 triglyceride	–0.143	>0.1
HDL_2/HDL_3 cholesterol ratio	-0.032	>0.1
LDL cholesterol	0.176	>0.1
LDL triglyceride	–0.033	>0.1

Rs: Spearman's rank correlation coefficient.

7.3.7. CPK levels and their relationship with IgM anti-BXO antibodies.

CPK levels were compared between MI patients and controls (Table 7.11), between 'deaths' and 'survivors' (Table 7.12), and between 'deaths' and controls (Table 7.13). CPK levels were significantly higher in MI patients compared to controls, and also in 'deaths' compared to controls. There were no significant differences between 'deaths' and 'survivors', although mean titres were higher in 'deaths'.

Table 7.11. Comparisons of CPK levels in MI patients and controls.

	MI patients (n=297)	Controls (n=146)	p value
CPK mean ± SEM (iu/l)	2107±106	154±11	<0.001

Table 7.12 Comparison of CPK levels in 'deaths' and 'survivors'.

	Deaths (n=88)	Survivors (n=227)	p value
CPK mean ± SEM (iu/l)	2048±194	1854±103	>0.1

Table 7.13. Comparison of CPK levels in 'deaths' and controls.

	Deaths (n=44)	Controls (n=74)	p value
CPK mean ± SEM (iu/l)	2112±305	126±9.2	<0.001

CPK levels were correlated with antibody levels in 'deaths', 'survivors' and controls, as individual groups and as one collective group (Table 7.14). There was a significant negative correlation between antibody levels and CPK levels in 'deaths'. No other significant correlations were observed in the other subject groups.

Table 7.14. Correlations between IgM anti-BXO antibody levels and CPK levels.

Antibody levels in the below groups	CPK
Deaths	Rs= –0.250 p=0.021 (n=88)
Survivors	Rs= 0.070 p >0.1 (n=227)
Controls	Rs= 0.015 p >0.1 (n=146)
Total (deaths, survivors, controls)	Rs= –0.026 p >0.1 (n=461)

Rs: Spearman's rank correlation coefficient.

7.4. DISCUSSION

In a preliminary study, MI patients were found to have significantly reduced antibody levels compared to controls. This result was unexpected in view of previous findings reported in the literature. Davies et al. (1969, 1974) and Oster et al. (1971) reported that levels of antibodies to whole dried cow's milk were elevated in patients who had suffered an MI. More recently, Harrison et al. (1990) reported that IgM anti-(XO) antibodies were significantly ($p<0.001$) raised in MI patients compared to controls.

In all of the aforementioned studies, the patients and controls were selected from a similar location and environment. Harrison et al. (1990) took blood samples from both MI patients and controls after their admission to a coronary care unit in hospital. The majority of the controls used in my initial study were from the Trowbridge community study, and the question arose as to whether titres in these controls differed from those used by Harrison et al. (1990). IgM anti-BXO antibody levels were accordingly compared in the two types of controls. Although the difference did not reach significance, the mean antibody titre was higher in the Trowbridge controls and the Dmax value was sufficiently large to suggest that with greater numbers this difference may have reached statistical significance.

Clearly, possible differences between types of control are important and an explanation was sought. A likely answer relates to differences in posture of the donors. Survey of the literature revealed that posture is known to affect the apparent concentration of blood constituents, altering the concentration of some indices by up to 15% (Fawcett et al.,1960; Dimmit, 1990). Changing from a horizontal position to a vertical position

increases the concentration of blood constituents because of an increase in hydrostatic pressure in dependant parts, resulting in a net movement of plasma water across the blood vessel wall. A reversal of posture results in a reversal of plasma water movement across the blood vessel wall. These changes, certainly for lipids, appear after 5 min and peak between 20-30 min (Tan et al., 1973). In the present study, the difference in mean antibody titres between the control groups was 17% which may well result from their different postures on blood sampling. Trowbridge controls were healthy volunteers who walked to the clinic and assumed a sitting position immediately prior to blood sampling. CCU controls, on the other hand, were lying down and had been doing so for many hours, as indeed, had the MI patients. Overall, it is clear that MI patients and CCU controls experienced essentially identical psychological effects and treatment prior to sampling and that the CCU controls are the most suitable for comparisons with MI patients.

In fact, IgM anti-BXO antibody levels in MI patients were found to be higher than those in CCU controls. Although this difference did not reach statistical significance, the Dmax value was 10.5%; 13.4% being needed for statistical significance. It is interesting to note that in a preliminary study comparing antibody levels in 50 MI patients with 50 CCU controls (results not shown), MI patients also had higher mean antibody titres than controls, with a Dmax value of 12%. IgM anti-BXO are consistently higher in MI patients, albeit marginally so, and although such an argument is far from statistical rigour, the impression is that larger subject numbers would show statistical significance. Certainly the data of Harrison et al. (1990) seem convincing and it may well be that with relatively small differences in titre between the two populations, chance in subject selection is a factor; a factor which will become less important as the populations increase in size, In this context, choice of controls and attention to their posture is clearly critical; a lesson that should perhaps be heeded in many clinical studies.

Patients who had died within 6 months of an MI had significantly raised levels of antibodies compared to patients who had survived at least 6 months. This finding is in agreement with Davies et al 1974, who, using a haemagglutination assay (see General Introduction, Chapter 1) found significantly higher levels of antibody to whole dried cows' milk in patients who had died within 6 months of a MI compared to survivors. Harrison et al. (1990) also noted higher levels of IgM anti-XO antibody in 12 patients who died within 6 months of an MI compared to 81 survivors, although not surprisingly, with such small numbers of subjects, differences did not reach statistical significance. In the present study, as expected 'deaths' had higher antibody levels than controls. Although this difference did not reach statistical significance, the Dmax value was greater than that found between

'deaths' and 'survivors', suggesting that larger numbers might produce statistical significance.

The findings of significantly higher IgM anti-BXO antibody levels in patients dying within 6 months of MI, compared with 'survivors' is important and potentially of prognostic value. Higher antibody levels did not reflect occurrence of previous MI (Table 7.4) and it would clearly be less interesting if higher antibody levels simply reflect a bigger MI. In order to address this possibility, correlations were sought between CPK levels and IgM anti-BXO antibody titres in MI patients. CPK is released into the bloodstream after myocardial cell death and serum levels are widely used as markers for occurrence and extent of MI. As expected, CPK were very much higher in MI patients compared to controls. Levels were also slightly higher in 'deaths' compared with 'survivors', albeit not significantly so. CPK levels showed no positive correlation with IgM anti-BXO antibody levels, indicating that the latter do not depend on the extent of the MI, and incidentally reinforcing the conclusions of Harrison et al. (1990) that IgM anti-BXO antibody levels are not a consequence of the MI itself. In fact, a negative correlation was found between CPK and antibody levels among the deaths; a finding that is difficult to explain. These antibodies, therefore, maybe the result of chronic lesions of the capillary endothelium exposing xanthine oxidase to the immune system, possibly over many months. The lack of positive correlation between CPK and antibody levels in MI patients supports the potential of IgM anti-BXO antibody levels as an independent indicator of survival chances following MI. It may well be that a routine assay of serum from MI patients would allow a threshold antibody level to be defined, above which special attention is indicated.

Indirect evidence suggests that the endothelium of hypercholesterolaemic vessels is a source of superoxide anions, probably via XO activation (Ohara et al., 1993). Because the association of atherogenesis and plasma levels of certain lipids/lipoproteins, it was of interest to seek correlations between such levels and those of IgM anti-BXO antibodies. Not surprisingly, plasma cholesterol concentrations were significantly higher in MI patients compared with controls, in line with the generally accepted association of raised cholesterol levels with increased risk of MI (Keys, 1980; Nichamen et al., 1975; Martin et al.,1986; Stamler et al., 1986; Shaper et al., 1986). Interestingly, levels, while higher in 'deaths' compared with controls, were not significantly so, possibly reflecting the smaller numbers of subjects involved (see above). It is also noteworthy, that levels of cholesterol, far from indicating risk of death in MI were actually lower in 'deaths' than in 'survivors'.

There were no significant correlations of antibody level with plasma and lipoprotein cholesterol and triglyceride levels in any of the subject groups. However it is interesting to

notice that in a group of 60 MI patients (Table 7.10), although there were no significant correlations, the strongest correlations (denoted by large Rs values) were with total cholesterol, LDL cholesterol and HDL_3 cholesterol. Total and LDL cholesterol are known risk factors for CHD because of their association with atherogenesis (Grundy, 1986). Although HDL cholesterol has an inverse relationship with CHD risk, this is due to reverse cholesterol transport via HDL_2. A low HDL_2/HDL_3 ratio is associated with angiographically defined coronary sclerosis (Miller et al., 1981). However in view of the weakness of these correlations and the lack of correlation with cholesterol in the other subject groups (Table 7.9), the association, if any, of these antibodies with lipids, lipoproteins and atherosclerosis is far from clear.

CHAPTER 8

ANTI-XANTHINE OXIDASE ANTIBODIES AND DIABETES MELLITUS

8.1. INTRODUCTION

In view of the possibility, discussed in Chapters 6 and 7, that levels of IgM anti-xanthine oxidase antibodies might reflect the presence and extent of chronic lesions of capillary endothelium, these levels could conceivably be higher in any disease group with a high risk of CHD.

Diabetes mellitus patients represent such a group and IgM anti-BXO IgM antibody levels were accordingly compared in diabetic patients and non-diabetic controls. Diabetic patients were subdivided into patients with NIDDM and IDDM and both diabetic patients and controls were subdivided into groups with and without CHD. Patients were from a study clinic in a community in Trowbridge (supplied by Dr.J.Lloyd, Royal United Hospital, Bath).

8.2. METHODS

8.2.1. Study population

Fasting blood samples were obtained from 187 diabetic patients and 187 non-diabetic subjects (Table 8.1).

	DIABETICS	CONTROLS
NUMBER	187 (98 male, 89 female)	187 (98 male, 89 female)
AGE (yrs) ± SEM	54.9± 0.87 (Range: 18-70)	55.4± 0.88 (Range: 18-70)

Table 8.1. Demographic data for diabetic patients and non-diabetic controls.

Comparisons of levels of IgM anti-BXO antibodies were made:

1. between diabetic patients (NIDDM, IDDM, males and females) and their age- and gender-matched non-diabetic controls.
2. between NIDDM and IDDM patients.
3. between subjects with and without CHD in both diabetic and non-diabetic patients.

8.2.2. Measurements

IgM anti-BXO antibodies were measured for each subject and expressed as a percentage of a standard serum (A1). The enzyme used for the ELISA was supplied by Biozyme Ltd.

8.2.3. Statistics

Comparisons involving anti-BXO IgM antibody levels were made using the Kolmogorov-Smirnov test. All values are mean ± SEM.

8.3. RESULTS

Comparisons of IgM anti-BXO antibodies between diabetic patients and controls are tabulated in Tables 8.2-8.7. There were no significant differences in antibody levels between diabetic patients (Table 8.2), NIDDM patients (Table 8.3), IDDM patients (Table 8.4) and their non-diabetic controls. However, mean antibody titres were considerably higher in IDDM patients compared to controls in both males and females. Table 8.5 shows that mean antibody titres were higher, although not significantly so, in IDDM patients compared to NIDDM patients. Fig. 8.1 illustrates the distribution profile for total IDDM and NIDDM patients.

Antibody levels were compared in diabetic and control patients with and without CHD (Tables 8.6 and 8.7 respectively). There were no significant differences between subjects with CHD and subjects without CHD.

Table 8.2. Comparison of anti-BXO IgM antibody levels between diabetic patients and controls.

	Total Diabetics	Total Controls	Male Diabetics	Male Controls	Female Diabetics	Female Controls
N	187	187	98	98	89	89
Age (Yrs) ±SEM	54.9±0.87	55.4±0.88	54.9±1.07	57.6±1.10	52.6±1.38	52.9±1.34
Titre ±SEM	36.7±1.70	38.7±2.29	34.0±2.08	33.4±2.28	39.7±2.72	44.5±4.02
Dmax	0.037		0.082		0.079	
P value	>0.1		>0.1		>0.1	

Titres are mean ± SEM (% of A1 standard).

Dmax is the maximum cumulative difference for this Table and subsequent Tables.

Table 8.3. Comparison of anti-BXO IgM antibody levels between NIDDM patients and controls.

	Total NIDDM	Total Controls	Male NIDDM	Male Controls	Female NIDDM	Female Controls
N	143	143	76	76	67	67
Age (Yrs) ±SEM	58.3±0.77	58.4±0.82	59.8±0.89	59.8±1.02	56.6±1.27	56.8±1.29
Titre ±SEM	33.6±1.81	38.3±2.86	31.8±2.27	33.2±2.78	35.5±2.86	44.0±5.17
Dmax	0.070		0.079		0.235	
P value	>0.1		>0.1		>0.1	

Titres are mean ± SEM (% of A1 standard).

Table 8.4. Comparison of anti-BXO IgM antibody levels between IDDM patients and controls.

	Total IDDM	Total Controls	Male IDDM	Male Controls	Female IDDM	Female Controls
N	44	44	22	22	22	22
Age (Yrs) ±SEM	43.7±1.97	45.6±2.05	47.1±2.81	49.1±2.90	40.3±2.64	41.3±2.67
Titre ±SEM	46.3±3.84	40.1±2.92	41.5±4.71	34.2±3.54	52.4±6.12	46.1±4.41
Dmax	0.182		0.227		0.136	
P value	>0.1		>0.1		>0.1	

Titres are mean ± SEM (% of A1 standard).

Table 8.5. Comparison of anti-BXO IgM antibody levels between NIDDM patients and IDDM patients.

	Total NIDDM	Total IDDM	Male NIDDM	Male IDDM	Female NIDDM	Female IDDM
N	34	44	19	22	15	22
Age (Yrs) ±SEM	46.1±1.35	43.7±1.97	49.3±1.60	47.1±2.81	42.1±1.86	40.3±2.64
Titre ±SEM	36.2±3.70	46.3±3.84	35.3±5.69	41.5±4.71	37.3±4.54	52.4±6.12
Dmax	0.205		0.191		0.273	
P value	>0.1		>0.1		>0.1	

Titres are mean ± SEM (% of A1 standard).

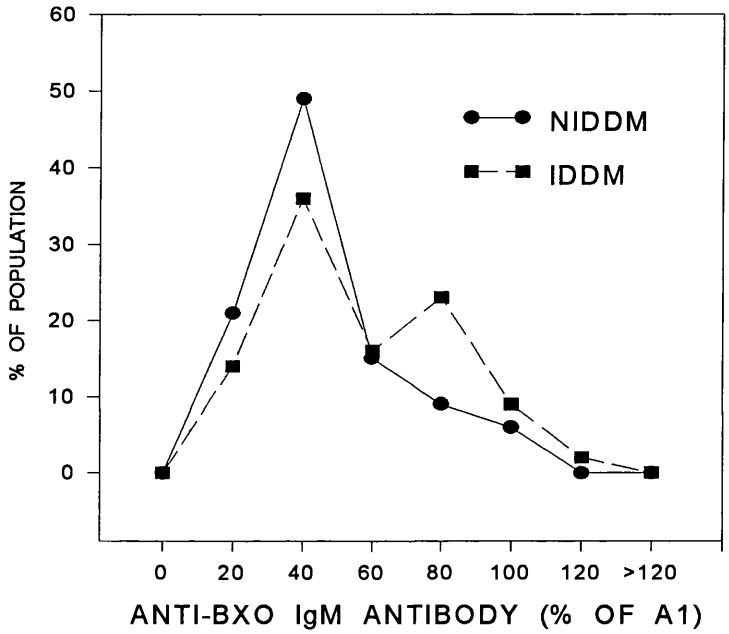


Fig. 8.1. Comparison of anti-BXO IgM antibodies between patients with NIDDM and IDDM. P>0.1.

Table 8.6. Comparison of anti-BXO IgM antibody levels between diabetic patients with and without CHD.

	Total CHD	Total No CHD	Male CHD	Male No CHD	Female CHD	Female No CHD
N	59	77	27	49	32	28
Age (Yrs) ±SEM	60.2±1.03	61.0±0.60	60.1±5.20	61.7±0.57	60.1±1.50	59.3±5.29
Titre ±SEM	30.2±2.49	34.6±2.62	30.4±2.50	32.8±2.56	30.1±2.95	37.6±5.28
Dmax	0.139		0.164		0.143	
P value	>0.1		>0.1		>0.1	

Titres are mean ± SEM (% of A1 standard).

Table 8.7. Comparison of anti-BXO IgM antibody levels between non-diabetic patients with and without CHD.

	Total CHD	Total No CHD	Male CHD	Male No CHD	Female CHD	Female No CHD
N	27	89	19	50	8	39
Age (Yrs) ±SEM	63.2±1.37	62.9±0.47	63.3±1.72	63.0±0.63	63.3±2.35	62.3±2.26
Titre ±SEM	32.8±4.45	36.8±4.00	34.3±5.64	32.2±3.55	29.4±7.18	42.6±7.86
Dmax	0.106		0.148		0.266	
P value	>0.1		>0.1		>0.1	

Titres are mean ± SEM (% of A1 standard).

8.4. DISCUSSION

Harrison et al. (1990) showed that elevated levels of anti-BXO IgM antibodies in MI patients persisted for several weeks after the infarction, suggesting that the antibodies were not due to the infarct itself, but produced in response to numerous immunisation events prior to the infarct. This finding was supported by the lack of correlation found between CPK levels, a marker for the extent of the MI, and antibody levels (Chapter 7). Diabetes is a major risk factor for morbidity and mortality from macrovascular disease, the prevalence of which is increased 3-4 fold (Garcia et al., 1974). It could follow that patients with diabetes, at greater risk of suffering a myocardial infarction, could be producing higher levels of anti-BXO antibodies in response to exposure of xanthine oxidase in atherosclerotic lesions.

Levels of anti-BXO IgM antibodies did not differ significantly between diabetic, patients and their age and gender matched controls. Low patient numbers and possibly small extent of atheroma in diabetic patients may account for differences not being comparable to those involving MI patients (Chapter 7). Mean antibody levels in male and female IDDM patients were considerably higher than age and gender matched NIDDM patients. However, this difference is unlikely to be due to CHD risk, since antibody levels were not raised in diabetic and non-diabetic patients with CHD. It may be due to self-immunisation of exposed enzyme as a result the destruction of β-cells in the pancreas by auto-antibodies, which can occur in IDDM.

CHAPTER 9

CONCLUSIONS

This section provides a general discussion with concluding remarks for each results Chapter.

Elevated fasting levels of triglyceride-rich lipoproteins were exaggerated and prolonged postprandially in hypertriglyceridaemic patients with NIDDM. This was in agreement with the finding that postprandial response was proportional to fasting triglyceride in both diabetic and control subjects. Raised concentrations of triglyceride-rich lipoproteins were particularly evident for chylomicrons and the large VLDL-1 subfraction. Not only was the proportion of large VLDL greater in NIDDM, but particle size within each VLDL subfraction was also larger, indicative of triglyceride-enrichment. Reduced levels of HDL and increased LDL-3 concentrations persisted postprandially in NIDDM, consistent with the known association between these indices and triglyceride. Low HDL cholesterol and raised levels of small, dense LDL are strong markers for CHD (Miller and Miller, 1987; Austin et al., 1988). Large VLDL is less readily catabolised than small VLDL (Stahlenhoef et al., 1984; Packard et al., 1984), thus providing less surface component for HDL production. In addition longer residence time of large VLDL allows cholesterol ester transfer with HDL and LDL by CETP in exchange for triglyceride (Tall, 1986). Hydrolysis of triglyceride in LDL results in the production of small, dense LDL (Auwerx et al.,1988; Levy et al.,1990; Zambon et al., 1993). Evidence of this cholesterol ester transfer process is provided by the triglyceride enrichment of HDL and LDL-2 in NIDDM as well as the predominance of LDL-3. Excess cholesterol ester transfer from HDL to triglyceride-rich lipoproteins also accounts for low HDL cholesterol levels in NIDDM. Instead of cholesterol ester returning to the liver via triglyceride-rich lipoproteins or HDL directly, it resides in VLDL, which now excessively cholesterol ester-enriched, is less readily catabolised and cleared by the liver, but more prone to removal by the scavenger pathway, thus leading to atherogenesis (Goldstein et al., 1980; Mahley et al., 1984).

Raised concentrations of insulin, NEFA and glucose were more apparent postprandially in NIDDM and are indicative of insulin resistance and relative insulin lack. This is consistent with the finding that β-cell function was reduced and insulin resistance was raised (although not significant) in these patients. Insulin resistance and relative insulin lack can lead to low LPL activity which would account for accumulation of triglyceride-rich

lipoproteins and HDL cholesterol. In addition, high levels of NEFA can stimulate hepatic triglyceride and VLDL synthesis (Sniderman et al., 1993).

While this study provides a detailed analysis of fasting and postprandial lipoprotein subfraction distribution and composition in NIDDM, investigations to assess the activities of LPL, HL and CETP would provide further insight to lipoprotein metabolism in NIDDM. Furthermore, a comparison between diabetic patients and non-diabetic patients matched for triglycerides would illustrate the effects of diabetes, independent of hypertriglyceridaemia, on lipoprotein metabolism. In addition, it would be interesting to determine the relative contribution of chylomicron remnants to the postprandial VLDL subfraction profile. Retinyl palmitate is incorporated into chylomicrons during absorption and therefore concentrations of retinyl palmitate provides a measure of chylomicrons and their remnants. This method has the disadvantage that retinyl palmitate may be exchanged with other lipoproteins (Krasinski et al., 1990). More recently, the determination of apo B48 using a specific monoclonal antibody has been developed (Peel et al., 1993).

Bezafibrate therapy reduced fasting and postprandial triglycerides and triglyceride-rich lipoproteins in NIDDM towards that of normolipidaemic subjects. This was particularly evident in the postprandial state, and in chylomicrons and VLDL-1, which decreased by 55% and 49% respectively. The proportion of small, dense LDL was reduced following bezafibrate therapy, consistent with reductions in large triglyceride-rich lipoproteins. Clearly these effects of bezafibrate reduce atherogenic potential in NIDDM. These effects are probably mediated by reductions in LPL activity (Vessby et al., 1980; Vessby et al., 1982; Heller et al., 1983; Eisenberg et al., 1984), NEFA concentrations (Alberti et al., 1990; Jones et al., 1990) and CETP activity (Homma et al., 1994).

There were no significant alterations in β-cell function and insulin resistance after bezafibrate therapy, although postprandial NEFA response was reduced, indicative of suppressed lipolysis. In addition, there were no changes in HDL and LDL cholesterol. An increase in HDL cholesterol would be consistent with a reduction in triglyceride-rich lipoproteins and furthermore, has been previously reported to be elevated following bezafibrate therapy in NIDDM (Seviour et al., 1988; Rovellini et al., 1992; Niort et al., 1992). Therefore, longer treatment time may have been required to have seen such an effect in the present study.

Although bezafibrate treatment resulted in substantial reductions in triglyceride-rich lipoproteins and small, dense LDL, these levels remained elevated compared to normolipidaemic non-diabetic subjects. Other means of improving dyslipidaemia include

diet and exercise. Weight reduction in NIDDM can reduce triglycerides and increase HDL cholesterol (Ginsberg and Grundy, 1982). Weintraub et al. (1988) showed that lipaemic response to an oral fat load containing mainly polyunsaturates was significantly lower than that caused by a fat load containing more saturated fats. Regular exercise has been reported to have beneficial effects on glucose levels, insulin sensitivity and dyslipidaemia in NIDDM (American Diabetes Association, 1990a; American Diabetes Association, 1990b). Short term exercise has been shown to enhance clearance of postprandial triglycerides (Annuzzi et al., 1987; Schlierf et al., 1987) and further improvements have been achieved by long term exercise (Merrill et al., 1989, Weintraub et al., 1989; Mankowitz et al., 1992). This effect has been shown to be mediated by elevated chylomicron lipolysis by LPL, rather than enhanced receptor-mediated uptake (Weintraub et al., 1989). Exercise-induced and diet-induced weight loss has also been shown to reduce the mass of small, dense LDL with a shift toward large, buoyant LDL particles (Williams et al., 1989; Williams et al., 1990).

Atherogenic abnormalities were found in the lipoprotein and lipoprotein subfraction profiles patients with SLE and psoriatic arthritis. Elevated proportions of LDL-3 in total LDL were found in female SLE and psoriatic patients, but not in males, and were not associated with raised triglyceride levels. HDL cholesterol was reduced in psoriatic patients and tended to be low in SLE patients compared to controls (although not significant). In addition to low HDL cholesterol levels, psoriatic patients tended to have lower serum and LDL cholesterol than controls, consistent with the findings of Lazarevic et al. (1992), whereas SLE patients tended to have raised serum and VLDL triglyceride, consistent with the findings of Ilowite et al. (1988). However there is uncertainty in SLE as to whether dyslipidaemia arises from disease activity or corticosteroid therapy (Ilowite et al., 1988; Leong et al., 1994). Predominance of small, dense LDL may be due mediators of inflammation affecting lipoprotein metabolism, since high percentage of LDL-3 in total LDL occured in both patient groups and was not associated with high triglyceride concentrations. Clearly further investigations with larger patient numbers are required to study the affect of gender and disease activity in both patient groups, and the affect of corticosteroid therapy in SLE patients, on LDL subfraction distribution and on the key enzymes and transfer proteins involved in lipoprotein metabolism.

In serum samples from normal healthy subjects, levels of IgM anti-HXO antibodies were higher than IgM anti-BXO antibodies suggesting that endogenous HXO rather than digested BXO is the immunogen. IgM anti-BXO and HXO antibody levels were higher in

females under 50 years compared to age-matched males and older females. Corresponding IgG levels were lower and showed no gender or age differences. Similar age and gender differences have previously been reported for IgM anti-BXO antibodies (Lewis and Ng, 1991), and the authors suggested that elevated levels in younger females may arise from cyclical reimmunisation of xanthine oxidase released during ischaemic events prior to menstruation.

IgM anti-BXO antibodies were raised in patients who died within six months of an MI compared to survivors, and tended to be higher in MI patients compared to controls (although not significant). Harrison et al. (1990) demonstrated that these antibodies were not raised as a consequence of the MI, but perhaps months prior to clinical manifestations. In line with this, in the present study, the extent of the MI (as judged by CPK levels) did not correlate positively with antibody levels. It may be that that IgM anti-XO antibodies are produced as a result of an immune response to exposed XO from chronic atherosclerotic lesions of the capillary endothelium. Thus, IgM anti-XO antibody levels could potentially serve as an independent marker of CHD and indicator of MI risk and survival chances following an MI. In view of the role of xanthine oxidase in ischaemia reperfusion injury, IgM anti-XO antibodies may play a protective role in neutralising XO from blood and tissues. A protective role for the antibody is consistent with younger women having lower incidence of CHD compared to age-matched males and older females (Heller, 1978).

Correlations of IgM anti-XO antibodies with certain lipids and lipoproteins were made because their association with atherogenesis. However, there were no such correlations in MI patients and controls.

In view of the possible association of IgM anti-XO antibodies with the presence and extent of lesions of the capillary endothelium, these levels were measured in diabetic patients, a disease group with a 3-4 fold risk of CHD. However, possibly due to small patient numbers and lack of extensive atheroma, there were no differences in antibody levels between diabetics and controls.

The finding of significantly higher IgM anti-XO antibody levels in patients dying within 6 months of MI compared to survivors is potentially of prognostic value as an indicator of survival chances. Routine assay of serum from MI patients would allow a threshold antibody to be defined, above which special attention is indicated. Differences in IgM anti-XO antibodies are very subtle and large patient numbers are required for population studies. A measure of immune complexes of XO and IgM anti-XO antibodies may be more revealing in terms of antibody response to XO.

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